Editor:

J. C. Stevenson, m.s.c.

Associate Editor: L. W. Billingsley, m.s.c.
Assistant Editor: R. H. Wiggins, b.sc.

Publication: K. L. MacIntyre, Marilyn Campbell, b.a.

Fisheries Research Board of Canada
Office of the Editor
116 Lisgar Street
Ottawa 4, Ont., Canada

Manuscripts for publication should be addressed to the Editor.

Application of the Bertalanffy Growth Equation to Problems of Fisheries Management: A Review

QH1
C192
c.2
v.26
Sept.-Dec.
1969

K. RADWAY ALLEN

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

ALLEN, K. RADWAY. 1969. Application of the Bertalanffy growth equation to problems of fisheries management: a review. J. Fish. Res. Bd. Canada 26: 2267–2281.

The development of large modern fisheries has led to the overexploitation, with resulting less than optimal production, of many of our fish stocks. A need to develop management techniques which would make it possible to hold stocks and exploitation rates more nearly at optimal levels has led to the development and extensive study of mathematical theory relating to fishing. An essential feature of this theory is the yield equation which relates the obtainable yield to such stock parameters as the number of recruits, rate of growth at various stages of life, natural mortality rate, and intensity of fishing. A widely used form of the yield equation is that developed by Beverton and Holt (U.K. Min. Agr. Fish. Food, Fish. Invest. 19: 1–533, 1957). In this equation the growth component is represented by the well-known Bertalanffy growth equation (Bertalanffy, Human Biol. 10: 181–213, 1938):

$$W_t = W_\infty (1-e^{-K(t-t_0)})^3.$$

It is not possible, however, to represent the growth of all fish satisfactorily by this equation, and where this is not possible, the Beverton and Holt yield equation also does not give satisfactory results. This situation calls for the need to develop a more generalized equation, using a more widely applicable form of the growth curve. As Bertalanffy (Helgolaender Wiss. Meeresuntersuch. 9: 5–37, 1964) has pointed out, the original equation assumes that catabolic processes are proportional to the weight of the animal, whereas anabolic processes are assumed to be proportional to the surface area or to the 2/3 power of the weight, but in practice at least the latter relationship frequently does not hold. The present paper examines the effect of different values of the exponent in the catabolic and anabolic components of the growth equation upon the shape of the growth curve and particularly upon the proportion of final size at which the point of inflection occurs. The modified yield equation embodying the generalized growth equation can be solved by means of tables of the incomplete beta-function by an extension of the method of Jones (Joint Meeting ICNAF/ICES/FAO, Lisbon, Doc. P21, 1957).

Received April 9, 1969

INTRODUCTION

ONE OF THE MOST SUCCESSFUL EXAMPLES of the application of mathematical techniques to the description of the biological processes involved in the management of a natural resource is found in the field of fisheries. Successful management requires the maintenance of the resource by the balancing of the catch against the additions to the stock from reproduction (less natural mortality) and growth. In recent years mathematical models have been developed which

Printed in Canada (J1438)

embody biologically meaningful descriptions of each of the processes involved and combine these descriptions to build up functions which can be used to assess the yield obtainable under various conditions and to forecast the effect of changes in exploitation, or in management practices. Growth being one of the principal components of this process, it has been necessary in building up these functions to use a representation of it which is biologically satisfactory and is also in a mathematical form suitable for inclusion in more complex models.

The growth curves formed on the principles originally suggested by Dr Ludwig von Bertalanffy[1] have proved to be particularly suitable for this purpose, and this paper is intended to describe their past use and to outline further developments which seem likely to make them still more widely applicable in the future.

From time immemorial, men in numerous parts of the world have drawn an important part of their sustenance from the fishes and other animals in the seas and fresh waters around them. For most of this time their efforts have had little effect, except in some restricted areas, upon the fish populations on which they preyed. In general, men have lived in equilibrium with their environment in this respect. Recently this situation has been changing. Fish catches have been increasing at an ever more rapid rate as the greater fishing power made possible by modern technological advances has helped to meet the growing demands of the rapidly multiplying human population. There are now few fish stocks, subjected to more than local fisheries, whose size and structure have not been seriously affected by fishing. In many instances the stocks have been driven below the level required to produce, on a sustained basis, the maximum yield of which they are capable.

Although restrictions, based on law or custom, and aimed at protecting fish during what seemed to be particularly vulnerable periods of their lives, have a long history in many human communities, it is only recently that informed opinion has seen any need for the protection of the fish stocks of the open seas. Just 100 years ago, in 1866, a British Royal Commission was able to report, "We advise that all acts of Parliament which profess to regulate, or restrict, the modes of fishing pursued in the open sea be repealed, and that unrestricted freedom of fishing be permitted hereafter." Even later, in 1883, no less an authority than T. H. Huxley could declare, "I believe then that the cod fishery, the herring fishery, the pilchard fishery, the mackerel fishery, and probably all the great sea fisheries are inexhaustible: that is to say that nothing we do seriously affects the number of fish. And any attempt to regulate these fisheries seems consequently from the nature of the case to be useless." But the fishing fleets were already growing in size and power, and the very meeting at which Huxley made this assertion passed a resolution calling for an international conference for the protection of fisheries by better regulation.

---

[1]This paper was originally prepared for, and will be reproduced in: Unity and diversity in systems: Essays in honour of Ludwig von Bertalanffy by Ronald G. Jones and George Brantl [ed.]. (In press.)

Since that time the continuous expansion of the world's fisheries has been accompanied by increasing efforts to regulate their operation so that the stocks can be maintained at a productive level. To provide a foundation for these regulations, there has been in most countries a progressive expansion of research on the stocks upon which the fisheries are based. Although much of this research has continued to be devoted to the biology — food, growth, movements, and so on — of the animals concerned, more and more attention has been given to quantitative aspects of the studies, to the actual sizes of the stocks, and to the measurement of the effects upon them of the catches taken by the fisheries. This has led to the development of a number of theoretical approaches aimed not merely at the measurement of existing stocks and conditions in quantitative terms, but also at forecasting the effects of possible changes in the nature of the fisheries themselves, and the determination of the conditions which would allow the maximum yield to be obtained on a sustained basis. These developments now represent one of the most successful examples of the application of mathematical techniques to biological problems.

BASIC THEORY OF FISHING

The basic principle on which the modern theory of fishing is based was probably first formulated in words by Petersen (1894) who stated, "For it cannot well be doubted that the same area of sea would be able to give a quantitatively greater profit as a constancy, when we suffered the stock of fish to be as fully developed, as in the years before the too eager fishing commenced,...and then took exactly so much as the stock could reproduce by new growth." In other words, that, for the level of yield to be maintained, the catch must be in equilibrium with the rate of production by the fish stock, and for this sustained yield to be a maximum the stock must be allowed to build up to an appropriate level.

The first attempt at a comprehensive mathematical formulation of the processes involved in the maintenance and exploitation of fish populations seems to have been that of Baranov (1918). The essential principles of his approach were those which still underlie the modern theory of fishing. He pointed out that the net production from the fish population, with which the yield must be in equilibrium, is the resultant of the processes of growth and mortality; and, by adopting mathematical models for these processes, he was able to set up differential equations from which the yield under various conditions could be computed. Unfortunately, Baranov's work was for long overlooked in most parts of the world, with the result that some time elapsed before these principles were re-enunciated and the developments began which have led to the theories of fishing most widely used at the present time. Baranov, in his analysis, also adopted as a model for the growth process simple linearity in length with equal annual increments throughout life. Although such a model may be adequate for dealing with events over a short period during the life of the fish, it is rarely a satisfactory description of the whole span of

later life, from the time the fish enter the fishery until they are caught or die naturally.

The basic principles involved were re-stated in a simple mathematical form by Russell (1931) in the equation

$$S_2 = S_1 + (A + G) - (C + M) \quad (1)$$

where S_1 and S_2 are the weights of the stock at the beginning and end of the year, A is the total of the initial weights of the recruits to the physical stock during the year, G is the amount added by growth of all the individuals which survived to the end of the year, and C and M respectively are the total weights of the catch and of those fish dying naturally. He pointed out that the stock would increase or decrease during the year according as (A + G) was more or less than (C + M).

A further advance was made by Graham (1935)who pointed out that the difficulties which arise in the application of Russell's equation from attempting to combine changes in numbers in the population with changes in the weights of individual fish disappear if logarithmic rates are used. He showed that Russell's equation, when written in the form describing the equilibrium situations

$$C = A + G - M \quad (2)$$

is mathematically correct if C, A, G, and M are all logarithmic rates. He further showed that this enabled deductions to be made regarding the values of some of these rates if the others are known or can be approximated.

THE YIELD EQUATION

Proceeding from this basis, Beverton and Holt (1957) (see also Beverton, 1953) developed a comprehensive theory of fishing centred round the yield equation

$$Y = FRe^{-M(t_{\rho'}-t_{\rho})} W_{\infty} \sum_{n=0}^{3} \frac{\Omega_n e^{-nk(t_{\rho'}-t_0)}}{F+M+nK} \left(1-e^{-(F+M+nK)}\right) \quad (3).$$

In developing this model, Beverton and Holt sought for a satisfactory means of representing mathematically each of Russell's four processes: recruitment (A), natural mortality (M), fishing mortality (C), and growth (G); and they then combined these into a single expression. They stressed the interdependence of these processes, which may be either direct (e.g., the same fish cannot both be caught and die naturally) or indirect (e.g., the rate of growth may be affected by the density of the population). They emphasized the resulting need for models which would not only describe the result of each process both accurately (e.g., would give a curve of suitable shape to describe growth) and in simple mathematical form, but that could also be related in their structure to the actual mechanisms involved, so that their parameters could be considered to be biologically meaningful.

The models used for recruitment, and natural and fishing mortalities, are of little direct concern to us here, and need be only briefly mentioned. The fishing and natural mortality rates were expressed in logarithmic terms, and in the basic model were regarded as having constant rates, F and M respectively. Thus the survivors of N_0 animals at time 0, subject to both fishing and natural deaths, would be given after time t by

$$N_t = N_0 e^{-(F+M)t} \quad (4).$$

The effects of changes in F and M were also considered; in particular F was regarded as being proportional to the fishing effort when this is expressed in suitable terms.

The model adopted for recruitment was based on the concept that a given number of recruits (R) enter the fishing area at age t_ρ, but that they are not subject to capture (e.g., they are able to escape through the meshes of the net) until they reach the age $t_{\rho'}$. During this interval they are subject to natural mortality at the standard rate M, so that the actual number of recruits on which the fishery operates is given by the expression

$$R' = Re^{-M(t_{\rho'}-t_\rho)} \quad (5)$$

which may be recognized towards the left-hand side of the yield expression. In later parts of their paper, Beverton and Holt considered the effect of changes in the values of M, $t_{\rho'}$, and t_ρ, and particularly of the situation which actually exists in most fisheries when the probability of capture increases gradually from zero to its maximum over a range of sizes or ages, and does not develop suddenly to its maximum value at a critical age, as the simple model implies.

THE GROWTH COMPONENT

Recruitment and mortality are relatively simple processes, although they may be subject to a variety of external and internal forces, and they may be represented effectively by these relatively simple mathematical models. Growth, however, is a much more complex process biologically, and its representation by a mathematical model, which, as well as fitting actual growth curves reasonably well, is also so constructed that its parameters can be regarded as biologically meaningful, is fraught with difficulty. Beverton and Holt, after considering and rejecting a number of possibilities such as linear and exponential growth, and the Gompertz and logistic curves, wrote, "The question is therefore whether a function can be found that not only gives a good and conveniently simple representation of data, but that can also be used for analytical studies of growth phenomenon.... The important question is not, however, whether a universal representation of growth in a mathematical form is possible, but whether a representation can be made that is adequate for a particular purpose, and in our opinion a function has been developed that satisfies the essential requirements of the present problem. This is the one developed by L. von Bertalanffy."

The function referred to is that described by Bertalanffy (1938, and numerous other papers). Starting from the assumption that growth is the resultant of anabolic and catabolic processes, and that these are allometrically related to the weight of the organism, the expression is obtained

$$\frac{dW}{dt} = HW^n - kW^m \tag{6}$$

where H and k are the coefficients of anabolism and catabolism respectively. If anabolism is governed by surface processes, and catabolism by mass processes, and if growth is isometric, then $n = 2/3$ and $m = 1$, so that

$$\frac{dW}{dt} = HW^{2/3} - kW \tag{7}.$$

Integrating, this leads to the growth equations

$$l_t = L_\infty (1-e^{-K(t-t_0)}) \text{ for length} \tag{8}$$

and

$$W_t = W_\infty (1-e^{-K(t-t_0)})^3 \text{ for weight} \tag{9}$$

where L_∞ and W_∞ are the asymptotic maximum length and weight, t_0 is the time at which the animal was theoretically at zero size, and $K = k/3$ in the original equation.

The function for growth in weight thus obtained is that used by Beverton and Holt in their yield equation, the cubed expression being expanded to give

$$W_t = W_\infty \sum_{n=0}^{3} \Omega_n e^{-nK(t-t_0)} \tag{10}$$

where Ω_n is a variable of summation having the values 1, −3, 3, −1 for $n = 0$, 1, 2, 3.

This expression also can be easily recognized as a component of the Beverton and Holt yield equation.

The derivation of the yield equation from these bases is comparatively simple. The total weight of the individuals of age t in a year-class is given, by extension from equations 4, 5, and 10, by

$$N_t W_t = R' e^{-(F+M)(t-t_{p'})} W_\infty \sum_{n=0}^{3} \Omega_n e^{-nK(t-t_0)} \tag{11}.$$

With an instantaneous rate of fishing mortality F, the rate at which the catch (Y_W) is being removed is given by

$$\frac{dY_W}{dt} = FN_t W_t \tag{12}$$

which from equation 11 with some rearrangement gives

$$\frac{dY_W}{dt} = FR'W_\infty e^{(F+M)t_{\rho'}} \sum_{n=0}^{3} \Omega_n e^{nKt_0 - (F+M+nK)t} \qquad (13).$$

Integrating between $t = t_{\rho'}$ and $t = t_\lambda$, where t_λ is the maximum age at which fish are caught and $\lambda = t_\lambda - t_{\rho'}$, and substituting for R′ from equation 5, then gives the yield equation 3. As calculated, this is the yield obtainable from a single year-class over the whole of its fishable life span, but, in a steady state, this is equal to the annual yield obtainable from the entire population.

This yield equation has proved a most valuable tool in the analysis of fisheries problems, and, as Beverton and Holt showed in their original publication, it is susceptible to modification to allow for a number of additional complexities not included in the simple statement given above. Probably its most valuable application has been to estimate for a given fishery what would be the effect on the yield of changing the conditions of the fishery, particularly as regards the age at first exploitation ($t_{\rho'}$) and the rate of fishing mortality (F). A particularly useful method of expressing results of this nature is by means of an isopleth diagram, first proposed by Beverton (1953), in which contours of equal yield are plotted against $t_{\rho'}$ and F, so that the effect on yield of any change in either or both variables may be seen at a glance. Figure 1, for example, shows such a diagram prepared by Dickie and McCracken (1955) for a flounder fishery in Nova Scotia. This approach has been used by numerous other authors for various fisheries. Application of this yield function to the analysis of particular situations has been facilitated by the production of tables giving values of the function for a range of values of the principal parameters (Beverton and Holt, 1964).

APPLICABILITY OF THE BASIC YIELD EQUATION

The success of this model depends, of course, on the accuracy with which its components fit the actual situation and, in particular, on the suitability of the basic Bertalanffy growth equation as a description of the growth of fishes in general. Beverton and Holt in their original study (1957) showed that it gave a good fit for a number of important commercial fishes, including plaice, haddock, and cod, and have since (1959) determined the parameters for Bertalanffy equations to fit published data for some 50 other species of fish, although apparently without critical examination of the goodness of fit. The equation has also been widely used by other workers to describe the growth of fish and shellfish (Dickie and McCracken, 1955; Southward and Chapman, 1965).

The suitability of the simple Bertalanffy growth equation as a component of yield calculations in a particular instance can be tested by examining the goodness of fit of the best obtainable curve to the actual growth data. It may also be examined more directly by comparing the results obtainable by means

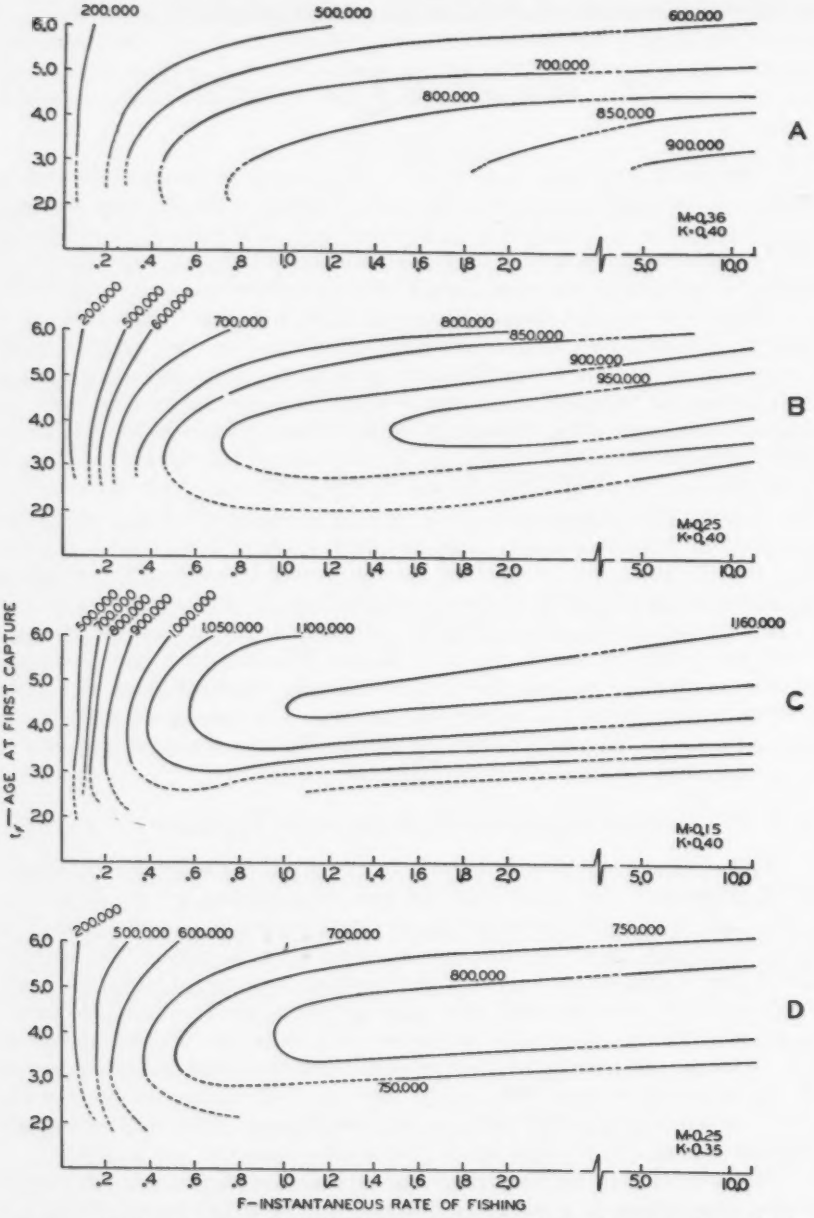

FIG. 1. Yield-isopleth diagrams for the flounder fishery of St. Mary Bay, Nova Scotia. (After Dickie and McCracken, 1955, fig. 3.) Yield contours are in units of pounds per 1 million recruits annually at age t_ρ = 3 years. Alternative diagrams are given for various possible combinations of the parameters M and K.

of the yield function with those produced by other methods which do not make assumptions as to the nature of the growth curve. For example, Allen and Forrester (1966) have shown that for the lemon sole in the Strait of Georgia, application of the yield function indicates that the equilibrium yield would decrease slightly if the size limit were increased from 30 to 32 cm, although application of a method which makes no assumptions about growth or mortality (Allen, 1953) showed that 32 cm was actually the optimum size limit and that the change from 30 to 32 cm must, therefore, produce a small increase in yield. The discrepancy in the estimated optimum size limit suggests that the Bertalanffy model in its simple form is not, in this instance, a sufficiently good description of growth during the fishable life span. A change in the natural mortality rate with age could also contribute to this discrepancy.

Thus, failure of the assumption that the simple Bertalanffy model adequately describes growth throughout the fishable life span may cause the Beverton and Holt yield equation to be unsatisfactory in some instances. Despite this, the Bertalanffy model may be applied to yield problems in other ways which involve only the much less restrictive assumption that the model describes growth simply in the range of possible ages of first recruitment to the fishable stock. Allen (1967) has, for instance, shown that with this assumption the ratio of the ultimate equilibrium yield (Y_2) obtainable with a new and higher size limit L_2, to the present yield (Y_1) with a limit L_1, is given by

$$\frac{Y_2}{Y_1} = \frac{Y_2}{Y_K} \cdot \frac{Y_K}{Y_1} \tag{14}$$

$$\text{where } \frac{Y_2}{Y_K} = \left(\frac{L_\infty - L_1}{L_\infty - L_2} \right)^{\frac{Z}{K} \cdot E'} \tag{15}$$

and L_∞ and K are the parameters of the Bertalanffy growth equation, Z is the total instantaneous mortality rate, E′ is the proportion ultimately caught of the fish reaching takeable size, and Y_K is the immediate catch with the new limit. $\frac{Y_K}{Y_1}$ can be simply estimated from the size distribution in the original catches.

MORE GENERALIZED GROWTH EQUATION

The occasions on which the simple Bertalanffy model either fails to give a good fit to the original growth data, or yields unsatisfactory results in the yield equations, also suggest the possibility of using a more generalized form of the equation. Bertalanffy himself has pointed out that although direct proportionality with the weight of the organism may be a satisfactory measure of the catabolic component in the basic equation 6, the position regarding

the anabolic component is much less definite. He has suggested (1964, and elsewhere) that the equation may therefore be written in the form

$$\frac{dW}{dt} = HW^n - kW \tag{16}$$

where n may represent the size dependence of appropriate metabolic rates. He has shown that n may have a wide range of possible values, if different metabolic processes are examined, and concludes (1964) that "We shall expect all sorts of allometric relationships of metabolic measures and body size — with a certain preponderance of surface or 2/3-power functions, considering the fact that many metabolic processes are controlled by surfaces. This is precisely what we find. In other words, 2/3 is not a magic number; nor is there anything sacred about the 3/4 power which more recently has been preferred to the classical surface law."

He has reviewed the values of n as derived from studies of metabolic rates, using oxygen consumption as an index, and has shown that insertion of these in equation 16 produces curves tending to correspond with the actual form of the growth curve of the organism. He has distinguished three principal types: (1) respiration surface-proportional, $n = 2/3$, growth curve as in equation 8, without inflection; (2) respiration weight-proportional, $n = 1$, growth exponential, no asymptote; (3) respiration intermediate, $2/3 < n < 1$, growth asymptotic but with an inflection. His conclusion that fish tend to belong to type (1) is confirmed, as a generalization, by the frequent good fit of fish growth data to the simple model that has already been noted.

The characteristics of the growth curves derived from equation 16 have been examined mathematically by various authors including Richards (1959), Chapman (1960), and Taylor (1962). Taylor, for instance, wrote the basic equation in the form

$$\frac{dW}{dt} = Hs - kW \tag{17}$$

where s = surface area, and he put $s = pl^a$, $W = ql^b$ so that n in equation 16 is equivalent to a/b in equation 17. Here s is the "physiological surface" rather than any actual surface of the organism. He then showed that this led to the equation for growth in length

$$l_t^{(b-a)} = L_\infty^{(b-a)} (1 - e^{-K(b-a)(t-t_0)}) \tag{18}$$

where $K = \frac{k}{b}$. This length growth equation may also be written in the form

$$l_t = L_\infty (1 - e^{-K(b-a)(t-t_0)})^{\frac{1}{b-a}} \tag{19}$$

and the corresponding weight growth equation is

$$W_t = W_\infty (1 - e^{-K(b-a)(t-t_0)})^{\frac{b}{b-a}} \tag{20}.$$

Substituting $a = 2$, $b = 3$ in these equations leads back to the simple Bertalanffy equations 8 and 9.

Richards (1959) and Taylor (1962) have both examined the influence of the parameter $[1/(b-a)]$, or its equivalent, on the shape of the growth curve. Richards has stressed the merits of this family of curves for the empirical fitting of growth data, and has suggested that, at least in some instances, the best fit may be obtained by making $(b-a)$ negative, although this requires that H and k should also be negative if the organism is to grow in the positive direction. This, however, requires the complete abandonment of the original concept of the basic equation as a representation of actual biological processes. It thus destroys one of the principal reasons why the Bertalanffy growth model was chosen as suitable for inclusion in Beverton and Holt's overall model of the processes of fisheries production and exploitation.

If these original concepts are to be retained, certain limits must be placed on the values which can be considered for b and a, and hence $(b-a)$. The value of b, which defines the relationship between length and weight, can be observed directly, although it is not without some complications in its interpretation (LeCren, 1951). The value is generally fairly close to 3.0, and would rarely be outside the limits 2.7–3.3. The value of the exponent relating metabolic rate to weight is almost invariably less than 1.0 and, as Bertalanffy has pointed out in the above quotation, lies rather commonly in the vicinity of 2/3 and is rarely much below this value. This exponent, which is n in equation 16, is equal to a/b, so that for the above range of values of b, the values of $(b-a)$ would range normally from zero to 1.1 and commonly from 0.9 to 1.1. Values lower than 0.9 and rather higher than 1.1 would also be expected to occur, although rarely.

THE SHAPE OF THE GROWTH CURVE

When $(b-a)$ is exactly equal to 1.0, the simple model applies as we have already seen, but if $(b-a)$ is between zero and 1.0, the resulting growth curve will be sigmoid with a point of inflection at a size given by

$$l_i = L_\infty \left[1 - (b-a)\right]^{\frac{1}{b-a}} \qquad (21).$$

The relative size at inflection increases as $(b-a)$ decreases. Taking $(b-a) = 0.75$ as a minimum value likely to occur naturally, the corresponding value of l_i is $0.158L_\infty$. The theoretical maximum value occurs when $(b-a) = 0$, and is L_∞/e $(= .368L_\infty)$. Although equation 21 is insoluble if $b - a = 0$, this result may be demonstrated by inserting a very small value, such as $(b-a) = .0001$, in it.

The curve for $(b-a) = 0$ is in fact identical with the Gompertz growth curve

$$l_t = L_\infty e^{-be^{-K(t-t_0)}}$$

as Richards (1959) has pointed out. Thus placing $n = m$ in equation 6 or $n = 1$ in equation 16 can be shown mathematically to lead to two growth curves

of entirely different form, the exponential, in which size increases ever more rapidly, and the Gompertz, which is asymptotic.

In the comparatively rare instances where $(b - a)$ is greater than 1.0, the growth curve is not inflected, and resembles the simple model in general form although it approaches the asymptote more slowly as $(b - a)$ increases.

Applying similar methods to the weight growth curve, equation 20, we find that inflection occurs at the weight

$$W_i = W_\infty \left[\frac{a}{b}\right]^{\frac{b}{b-a}} \tag{22}.$$

Since a/b equals n of equation 16 this becomes

$$W_i = W_\infty n^{\frac{1}{1-n}} \tag{23}.$$

The usual range of values of n has been seen to be from 2/3 to 1. The corresponding range of W_i/W_∞ is .296–.368.

Thus growth curves based on the more generalized Bertalanffy model, and using values of the parameters in accordance with the biological basis of the model, may be expected to be, for length, generally uninflected, or, rarely, with an inflection at less than 15% of the asymptotic length; and, for weight, generally inflected, with the point of inflection at about 30–38% of the asymptotic weight. These general characteristics would, in fact, apply to the growth curves of a great many species of fish.

Although the simple model has been very widely used in fisheries work, and has been fitted to numerous sets of data, there have been relatively few attempts to determine the parameters of the more generalized equation. Taylor (1962) examined published data for several species of fish using approximate trial-and-error methods. For Arctic char (data from Grainger, 1953) he found $(b - a) = 0.3$; and for rainbow trout (Hasler, 1938) 0.4. Southward and Chapman (1965) have applied more exact methods, using a computer, to data for Pacific halibut, walleye, and largemouth bass. They found that the values of the parameters varied significantly with the range of age for which the analysis was performed. For halibut the most probable value of their parameter m varied from 0.60 to 0.78. Since $(1 - m)$ in their notation equals $(b - a)$ in the present paper, this corresponds to values of $(b - a)$ of 0.22–0.40. For walleye and bass, the values were respectively, m, 0.54–0.38, 1.20–0.59; $b - a$, 0.46–0.62, −0.20–0.41.

Since Southward and Chapman also state that the exponent of the weight–length relationship (b) for halibut is about 3.2, one may estimate $a(=3.2 - 0.22$ to $0.40)$ as 2.98–2.80. Thus a/b, which equals n in the basic equation 16, appears to lie between .87 and .93, which is within the range discussed earlier as being biologically reasonable.

A GENERALIZED YIELD EQUATION

If the Bertalanffy growth equation, when generalized in this way, is still to be applied to problems of fisheries management, corresponding modifications

must be made in the yield equation. The original equation, as proposed by Beverton and Holt, was based on the simple Bertalanffy model, and in effect assumed that the exponent $[b/(b-a)]$ in equation 20 was equal to 3. Using the integral value for this parameter made it possible to use an expansion in constructing the yield equation 3. When the generalized equation is used, without this restriction on the value of the exponent, it is necessary to replace the expansion by an integral function and the equation becomes

$$Y_W = FRe^{-M(t_{\rho'}-t_\rho)}\, W_\infty \int_{t_{\rho'}}^{t_\lambda} e^{-(F+M)(t-t_{\rho'})} \left[1-e^{-K(t-t_0)}\right]^{\frac{b}{b-a}} \cdot dt \qquad (24)$$

Fortunately, this integral can be reduced to a standard form which has been tabulated. Jones (1957) has shown that by substituting

$$y = e^{-K(t-t_0)}$$

and

$$x = e^{-K(t_{\rho'}-t_0)}$$

$$x_1 = e^{-K(t_\lambda-t_0)}$$

the equation becomes

$$Y_W = FRe^{-M(t_{\rho'}-t_\rho)}\, W_\infty\, e^{(F+M)(t_{\rho'}-t_0)} \cdot \frac{1}{K} \int_x^{x_1} y^{\frac{F+M}{K}-1} (1-y)^{\frac{b}{b-a}} \cdot dy \qquad (25)$$

and that this integral is the difference between two incomplete beta-functions. This enables us to write

$$Y_W = FRe^{-M(t_{\rho'}-t_\rho)}\, W_\infty\, e^{(F+M)(t_{\rho'}-t_0)} \left\{ Bx\left(\frac{F+M}{K}, \frac{b}{b-a}+1\right) - Bx_1\left(\frac{F+M}{K}, \frac{b}{b-a}+1\right)\right\} \qquad (26)$$

where $Bx\left(\frac{F+M}{K}, \frac{b}{b-a}+1\right)$ is the incomplete beta-function with parameters x, $\frac{F+M}{K}$, and $\frac{b}{b-a}+1$. The values of these functions are available in tables, thus enabling yield calculations to be made relatively simply within the limits of the tables. Extensive tables were published by Pearson (1948) but were not designed for fisheries use. Jones (1957) published a short table intended for this purpose but limited to the simple Bertalanffy equation, the only value of $\left(\frac{b}{b-a}+1\right)$ given being 4.0. Wilimovsky and Wicklund (1963) have,

however, published quite extensive tables designed for fisheries purposes, using values of $\left(\frac{b}{b-a} + 1\right)$ from 3.50 to 4.50 with intervals of 0.125.

In presenting their tables, both Jones (1957) and Wilimovsky and Wicklund (1963) have pointed out the advantage of using the incomplete beta-function both in simplifying the calculations and also in enabling them to be made when growth is allometric, so that the exponent of the weight–length relationship is not exactly 3.0. It has been shown here, however, that the more generalized basic equation 16 leads to both length and weight growth equations of the same general type, which also includes the weight growth equation derived from the simple Bertalanffy model, in which the term $(1-e^{-k(b-a)(t-t_0)})$ has an exponent. The incomplete beta-function technique, therefore, allows yield calculations to be made, provided tables for the appropriate value of the exponent are available, for all growth models based on equation 16 whatever the value of n, and whether growth is isometric or allometric.

Thus, it can truly be stated that the mathematical descriptions of the growth curves of animals which have been proposed by Dr L. von Bertalanffy have played an important part in the development of the modern theoretical approaches to the problems of fisheries management. In their simplest form they are an essential component of the most widely used function for calculating the yield obtainable from a fish population under various conditions. The use of somewhat more complex growth curves developed from the basic principles laid down by Bertalanffy will probably enable the yield function to be modified to give satisfactory results in an even wider range of situations. Some of the directions in which these modifications may be expected to develop have been outlined in the present paper.

REFERENCES

ALLEN, K. R. 1953. A method for computing the optimum size limit for a fishery. Nature 172: 210.

1967. Some quick methods for estimating the effect on the catch of changes in the size limit. J. Conseil Conseil Perm. Intern. Exploration Mer 31: 111–126.

ALLEN, K. R., AND C. R. FORRESTER. 1966. Appropriate size limits for the lemon sole (*Parophrys vetulus*) in the Strait of Georgia. J. Fish. Res. Bd. Canada 23: 511–520.

BARANOV, F. I. 1918. On the question of the biological basis of fisheries. Nauchn. Issled. Iktiol. Inst. Izves 1: 81–128.

BERTALANFFY, L. VON. 1938. A quantitative theory of organic growth (inquiries on growth laws. II). Human Biol. 10: 181–213.

1964. Basic concepts in quantitative biology of metabolism. Helgolaender Wiss. Meeresuntersuch. 9: 5–37.

BEVERTON, R. J. H. 1953. Some observations on the principles of fishery regulation. J. Conseil Conseil Perm. Intern. Exploration Mer 19: 56–68.

BEVERTON, R. J. H., AND S. J. HOLT. 1957. On the dynamics of exploited fish populations. U.K. Min. Agr., Fish. Food, Fish. Invest. 19: 1–533.

1959. A review of the lifespan and mortality rates of fish in nature, and their relation to growth and other physiological characteristics. Ciba Found. Symp. Lifespan Animals: 142–177.

1964. Tables of yield functions for fishery assessment. FAO Fish. Tech. Paper 38: 1–49.

CHAPMAN, D. G. 1960. Statistical problems in dynamics of exploited fisheries populations. Proc. Fourth Berkeley Symp. Mathematical Statistics Probability: 153–168.

DICKIE, L. M., AND F. D. MCCRACKEN. 1955. Isopleth diagrams to predict equilibrium yields of a small flounder fishery. J. Fish. Res. Bd. Canada 12: 187–209.

GRAHAM, M. 1935. Modern theory of exploiting a fishery and application to North Sea trawling. J. Conseil Conseil Perm. Intern. Exploration Mer 10: 264–274.

GRAINGER, E. H. 1953. On the age, growth, migrations, reproduction potential and feeding habits of the Arctic char (*Salvelinus alpinus*) of Frobisher Bay, Baffin Island. J. Fish. Res. Bd. Canada 10: 326–370.

HASLER, A. D. 1938. Fish biology and limnology of Crater Lake, Oregon. J. Wildlife Management 2: 94–103.

JONES, R. 1957. A much simplified version of the fish yield equation. Joint Meeting ICNAF/ICES/FAO, Lisbon. Doc. P21. p. 1–8.

LECREN, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J. Animal Ecol. 20: 201–219.

PEARSON, K. 1948. Tables of the incomplete Beta-function. Univ. Press, Cambridge, England. 494 p.

PETERSEN, C. G. J. 1894. On the biology of our flatfishes and on the decrease of our flatfish fisheries. Rept. Dan. Biol. Sta. 4: 1–47.

RICHARDS, F. J. 1959. A flexible growth function for empirical use. J. Exptl. Botany 10: 290–300.

RUSSELL, E. S. 1931. Some theoretical considerations on the "over-fishing" problem. J. Conseil Conseil Perm. Intern. Exploration Mer 6: 3–20.

SOUTHWARD, G. M., AND D. G. CHAPMAN. 1965. Utilization of Pacific halibut stocks: study of Bertalanffy's growth equation. Rept. Intern. Pacific Halibut Comm. 39: 1–33.

TAYLOR, C. C. 1962. Growth equations with metabolic parameters. J. Conseil Conseil Perm. Intern. Exploration Mer 27: 270–286.

WILIMOVSKY, N. J., AND E. C. WICKLUND. 1963. Tables of the incomplete Beta-function for the calculation of fish population yield. Univ. British Columbia, Vancouver, B.C. 291 p.

Physiological Tolerances and Behaviour Responses of Five Species of Haustoriidae (Amphipoda: Crustacea) to Five Environmental Factors[1]

D. D. SAMEOTO[2]

Department of Biology
Queen's University, Kingston, Ont.

SAMEOTO, D. D. 1969. Physiological tolerances and behaviour responses of five species of Haustoriidae (Amphipoda: Crustacea) to five environmental factors. J. Fish. Res. Bd. Canada 26: 2283–2298.

Five species of haustoriid amphipods from Cape Cod, Massachusetts, differed in their tolerances to high temperature, desiccation, low salinity, and low oxygen concentration and in preferences for different sediment sizes. Two intertidal species, *Haustorius canadensis* Bousfield and *Neohaustorius biarticulatus* Bousfield, survived longer in air at 100% relative humidity and were more resistant to high water temperature than three species that are basically subtidal, *Acanthohaustorius millsi* Bousfield, *Parahaustorius longimerus* Bousfield, and *Protohaustorius deichmannae* Bousfield. All five species preferred sediments of a particular size range, the intertidal species selecting coarser sediments than the subtidal species. The sediment preference shown by the intertidal species could be changed by altering the organic conditions of the sediment by either drying or igniting the sediment. *Acanthohaustorius millsi* was the species most tolerant to low oxygen concentration and *H. canadensis* had the lowest oxygen consumption rate of the five species.

The ecological significance of these findings is discussed in relation to the distribution of the five species on Cape Cod.

Received December 6, 1968

INTRODUCTION

TWO INTERTIDAL SPECIES of haustoriid amphipods, *Haustorius canadensis* Bousfield and *Neohaustorius biarticulatus* Bousfield, were vertically and horizontally separated when they occurred in the same environment on Cape Cod, Massachusetts, and were seldom found subtidally (Sameoto, 1969a). The haustoriid species *Acanthohaustorius millsi* Bousfield, which is mainly subtidal, was found in the same region, but seldom overlapped in distribution with the intertidal species and was rarely collected in the intertidal zone. Two other species found on Cape Cod, *Parahaustorius longimerus* Bousfield and *Protohaustorius deichmannae* Bousfield, were never found in the same type of habitat as the intertidal species. *Acanthohaustorius millsi*, *P. longimerus*, and *P. deichmannae* are basically subtidal, but occasionally are found in water-

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy from Queen's University, Kingston, Ont.

[2]Present address: Fisheries Research Board of Canada, Marine Ecology Laboratory, Bedford Institute, Dartmouth, N.S.

Printed in Canada (J1303)

saturated, intertidal sands (Bousfield, 1965; Croker, 1967; Dexter, 1967). Sameoto (1969a, b) suggested that five environmental factors were important in limiting the distributions of these five species: the maximum temperature the sediment reached during low tide, exposure to interstitial air in the sands, low salinity, the grain size and organic content of the sediment, and low oxygen conditions of the interstitial waters of the sands.

The intertidal sands containing the intertidal species reached temperatures of 31 C during the summer, whereas the maximum recorded temperature of sand containing the subtidal species was 27 C (Sameoto, 1969a, b). The intertidal amphipods were exposed to air with a relative humidity greater than 80% while trapped in sands above the water table at low tide (Croker, 1967), and to salinities as low as 20‰ when heavy rains occurred at low tide (Sameoto, 1969a). Differences were found in grain size and organic content between the sands containing intertidal and subtidal amphipods. The abundance and distribution of the amphipods were affected by the presence of black anaerobic sand, with few animals present in sand with low oxygen conditions (Sameoto, 1969a, b). It was not possible to measure the oxygen concentration of the waters in the sands directly; therefore, an index of the oxygen requirements of the haustoriids was obtained by measuring their lethal oxygen levels as well as their rates of oxygen consumption.

This paper describes the responses of five haustoriid species in experiments dealing with the five environmental factors, with a view to relating the species responses to the differences in distribution shown by the subtidal and intertidal species.

METHODS AND MATERIALS

Specimens of *H. canadensis* and *N. biarticulatus* used in all the experiments were taken from Sippewissett Creek (Sameoto, 1969a); *A. millsi*, *P. longerimus*, and *P. deichmannae*, were taken from Barnstable Harbor (Sameoto, 1969b). Only similar sized adults of each species were used in the experiments.

Survival at High Temperature

Acanthohaustorius millsi, *P longimerus*, and *P deichmannae* were collected when the low tide water temperature was 20 C, and *H. canadensis* and *N. biarticulatus* were taken from water at 22 C. The animals were held in water at 25 C and 31‰ for 48 hr before the experiments were carried out. Ten animals of each species were placed in a 400-ml beaker containing 200 ml of sea water at a salinity of 31‰. The beaker was placed in a water bath at 25 C and hot water was added to the bath at a rate that heated the sea water in the beaker 1 degree C per min. Temperature was increased until all the animals were still and no signs of life seen. The animals were held at this temperature for 3 min then transferred to sea water at 25 C and held for 1 hr to detect recoveries. The experiments were repeated three times for each species.

Survival in Air at 100% Relative Humidity

All the animals used in the tests were taken from water at 20 C and held for 2 weeks in an aquarium at 25 C before being used. Thirty animals of each species were blotted on filter paper to remove any free water. They were then put on a disc of filter paper and placed in a 20-ml beaker with six animals per beaker, and the beaker was placed in a 16-oz jar containing 20 ml of water. A wire, which was used to prod the animals to see if they were alive, was passed through

the cap of each jar, and the hole was sealed with plasticine. The jars were tightly sealed and placed in an incubator at 25 C. Observations on the animals were made at 1-hr intervals to determine the number of animals surviving.

Survival at Low Salinities

Specimens of all species except *A. millsi* were collected in June when the high- and low-tide water temperatures at Barnstable Harbor ranged between 10 and 17 C and those at Sippewissett Creek ranged between 14 and 20 C. The animals were held in water at the experimental temperature of 15 C for 48 hr before the experiment. All tests were run at this temperature. Specimens of *A. millsi* were collected in August when the water temperatures at high and low tide ranged between 15 and 22 C. Since adequate cooling facilities were not available, tests on this species were conducted at 20 C.

The test salinities were obtained by diluting natural sea water with distilled water, the determinations being made with a conductivity meter. Salinities used were 31‰ (control), 25‰, 20‰, 15‰ 10‰, 5‰, 2.5‰, and distilled water (0‰). Thirty specimens of *P. deichmannae* and 10 specimens of each of the other four species were placed in 800 ml of water in 1000-ml beakers, and the beakers were held in a water bath with a temperature control accuracy of ±0.5 C. The beakers were continuously aerated. Observations on the animals were made at ½-hr intervals and all animals believed to be dead were removed with a pipette, transferred to 31‰ water, and left for 3 hr to detect any recoveries.

Sediment Preference

The sand used for all experiments unless otherwise stated was taken from Sippewissett Creek.

All five species were given the choice of four different sizes of sand that had been sorted using Tyler screens while the sand was wet. The grain sizes were determined with the Woods Hole rapid sediment analyzer (Zeigler et al., 1960). The median sizes of the sands were 1.50, 0.817, 0.360, and 0.283 mm. A portion of each sand was placed in one of four quarters of a petri dish (9.5-cm diam) that had been divided with strips of plastic. The dishes were placed into 1000-ml beakers wrapped in black masking tape to prevent the entry of light from underneath. Unfiltered sea water was carefully added so as not to disturb the sand. To each of the beakers were added 16–25 animals of one species, which were allowed to select the size of sand that they preferred. The water was continuously aerated near the surface in a way that did not disturb the sand. During the night the beakers were covered with a black cloth so the animals were not disrupted by any light. The water was siphoned out of the beakers after 48 hr, the sand of each compartment was screened, and the animals were removed and counted. Only animals that were alive and in the sand were recorded.

Only *H. canadensis* and *N biarticulatus* were used in the following experiments. Except as indicated, these were conducted in the same manner as the one just described.

Sand dried for 48 hr at 40 C was screened into four fractions with median sizes of 0.540, 0.375, 0.308, and 0.228 mm. Each of the four sizes of sand was tested for the presence of plant pigment and for organic content by the methods described by Sameoto (1969a, b). Sand of each size was placed in one of four compartments of the petri dishes and the species were allowed to select the sands they preferred.

The animals were then given the choice of the above four types of sand after the sands had all their organic matter removed by ignition at 500 C for 72 hr. The numbers of animals of each species in each of the sand sizes were compared with an analysis of variance and Duncan's new multiple range test (Steele and Torrie, 1960).

Unsorted sand dried at 40 C for 48 hr and untreated sand were placed in opposite compartments of the petri dishes so that half of the dish was filled with each of the two types of sand. The animals were allowed to select the type of sand they preferred. The numbers of animals found in the two types of sand were compared by a paired Student's *t*-test. The same statistical test was used in all of the following experiments.

Unsorted sand was dried at 40 C for 48 hr then soaked in filtered sea water for 5 days at 25 C. The sand was mixed each day to prevent anaerobic conditions from forming. This treated sand

was placed in opposite sections of the petri dish with untreated sand in the other sections of the dish. The animals were allowed to select the type sand they preferred.

Sand ignited at 500 C for 72 hr was placed in opposite sections of the petri dish from untreated sand and the animals allowed to select between them.

The animals were then given the opportunity to select between sand ignited at 500 C and sand dried at 40 C. The two sands were placed in the petri dishes in the same manner as the pairs of sands in the earlier experiments.

Untreated sand from Sippewissett Creek, where the intertidal species are common (Sameoto, 1969a), and sand from Nobska Beach, where only the subtidal species are present (Sameoto, 1969b), were placed in opposite sections of the petri dishes, and the two species were given a choice between these two sediments. The sand from Nobska Beach was finer (mean size = 0.256 mm) and had a higher organic content (0.250% by weight determined by ignition) than sand from Sippewissett Creek (mean size = 0.364 mm, and organic content = 0.193% by weight).

To exclude the influence of the organic matter on the selection of the sediments from the two above locations the sands were ignited at 500 C to remove all the organic material. A test was then conducted as above using the sands without organic matter.

Experiments similar to those above were conducted with sand from Barnstable Harbor, another area that contains only the subtidal species (Sameoto, 1969b), and sand from Sippewissett Creek. The mean size of the sand from Barnstable Harbor was 0.271 mm and the organic content of the sand was 0.300% by weight. The animals were given a choice between untreated sands from the two locations. Then the sands were ignited at 500 C and the experiment repeated using the sands without their organic matter.

Low Oxygen Tolerance and Rates of Oxygen Consumption

The procedure followed to measure low oxygen tolerance and rates of oxygen consumption was modified from Conover (1960). Filtered sea water (31‰) containing 50 mg of streptomycin sulfate per liter was allowed to stand at room temperature for 24 hr. The oxygen concentration of the water was determined by the modified Winkler method (Strickland and Parsons, 1965) with concentrated HCl being substituted for concentrated H_2SO_4. Six animals of similar size, held for 24 hr in water at 25 C with 4 ml oxygen/liter, were blotted on filter paper to remove water from the holding container, then placed in 35-ml bottles with the prepared sea water. At the same time three control bottles for each experimental bottle were prepared in the same manner, but without the animals. The bottles were held at 25–26 C for the entire experiment. The experiment ended when 50% of the animals were considered dead. The criterion for determining death was the absence of any movement of the appendages for 5 min. At the end of the experiment the oxygen concentration of the water of each experimental bottle, as well as that of the controls, was determined. When the sample of water was removed, the animals were placed in air-saturated water to determine if any of the animals considered dead would recover. The animals were then blotted on filter paper, placed on teflon discs, dried at 70 C for 72 hr, and weighed.

The oxygen concentration was calculated by the equation given by Conover (1956). The amounts of oxygen used by the animals were taken as the average differences in oxygen concentration between the experimental and the control bottles. The average rate of oxygen consumption during the experiments was calculated as:

$$\mu\text{liters of } O_2\text{/gram dry weight per hour} = \frac{A}{W \times T} \times 1000$$

where A = amount of oxygen used in milliliters; W = dry weight of animals in grams; and T = duration of experiment in hours.

The CO_2 concentration increased a small amount, as was seen by a drop in *p*H from 7.05 in the controls to 7.02 in the experimental bottles. Thomas (1954) found that a drop in the *p*H from 8.5 to 7.0 due to CO_2 concentrations caused no permanent response in the ventilation rate of the gills of *Homarus vulgarus*. Therefore, the small decrease in *p*H in this experiment was ignored.

DETERMINATION OF THE GILL AREA

The gill area of each species was measured to determine if there was any correlation between the area and the rate of oxygen consumption or the low lethal oxygen concentration of each species.

The gills from one side of the animal were removed and stained with an alcohol and iodine solution. Then the image of each gill was produced with a projecting microscope and a drawing of the gill made. The area of the gills was determined from the drawings of five gills by using a planimeter. The area obtained from the drawings was multiplied by four to get the total gill area, since only one of the two sides of the gill surface was measured and only half of the gills were measured. The thickness of the gills was ignored in the calculations as the gills of all the species are smooth and leaflike. The body length of each animal was measured as described in Sameoto (1969a). Body weights for each species were then determined from body weight:body length curves (Sameoto, 1967, 1969b).

RESULTS

Survival at High Temperature

The intertidal species, *H. canadensis* and *N. biarticulatus*, had 100% mortality at 41 C. When the animals were subjected to water of 39 C for 3 min, then placed in water at 25 C, all specimens of both species survived. The subtidal species, *A. millsi*, *P. longimerus*, and *P. deichmannae*, had a 100% lethal temperature of 36 C. All specimens of these species survived a temperature of 35 C for 3 min and recovered when placed in 25 C water immediately after they were taken from the 35 C water. Under the experimental conditions described, the temperatures that would kill 50% of the animals of these species must be very close to the temperatures that caused 100% mortality.

This experiment did not determine the absolute upper lethal temperature as defined by Fry et al. (1942) or McLeese (1956), and other lethal temperatures would have been found if the animals had been acclimated to high temperature and the rates of temperature increase during test exposures had been different (Sprague, 1963). But these values do give an indication of the relative tolerances to high temperature by the species.

Survival in Water-Saturated Air

The maximum survival times for *H. canadensis* and *N. biarticulatus* were much greater than those for *A. millsi*, *P. longimerus*, and *P. deichmannae* (Fig. 1). All the specimens of *H. canadensis*, 85% of *P. longimerus*, 65% of *N. biarticulatus*, 58% of *A. millsi*, and 20% of *P. deichmannae* survived for 24 hr. *Neohaustorius biarticulatus* had a 50% mortality rate for the first 48 hr, after which time no aminals died until 120 hr. *Neohaustorius biarticulatus* showed a curvilinear survival curve; the other species showed relatively linear survival curves.

Survival at Low Salinities

In a salinity of 10‰ after 96 hr the mortality rates of the species were *H. canadensis*, 0%; *A. millsi*, 10%; *N. biarticulatus*, 25%; *P. longimerus*, 30%; *P. deichmannae*, 100% (Fig. 2). But at salinities below 10‰ *N. biarticulatus*

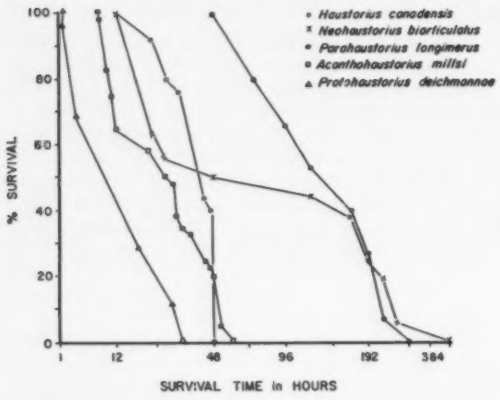

FIG. 1. Survival times of five species of haustoriids in water-saturated air at 25 C.

had a lower mortality rate than *A. millsi*. For the entire range of salinities the species fell in descending order of survival as follows: *H. canadensis*, *N. biarticulatus*, *A. millsi*, *P. longimerus*, and *P. deichmannae*. No mortalities occurred at a salinity of 25‰ in any of the species.

Mortality occurred in *P. longimerus* at 20‰ but not at 15‰, possibly because of factors other than salinity as 20% of the animals held in the control salinity (31‰) died after 96 hr. Because of the scarcity of this species at Barnstable Harbour it was not possible to repeat the experiment. None of the other species had mortalities at a salinity of 31‰.

SAND PREFERENCES

When the species were provided with a choice between different sizes of sand that had been screened while wet, *H. canadensis* and *N. biarticulatus* preferred ($P \leq 0.05$) sands with median sizes of 0.817 and 0.360 mm; *A. millsi* and *P. deichmannae* preferred sands with median sizes of 0.360 and 0.283 mm (Table 1). An analysis of variance showed no significant difference in the numbers of *P. longimerus* in sands with median sizes of 0.817, 0.360, and 0.283 mm, but they apparently avoided sands of 1.50 mm.

The experiment with sorted sands that had been first dried at 40 C showed differences in the numbers of *H. canadensis* ($F = 7.14$, df = 3, 84; $P < 0.01$) and *N. biarticulatus* ($F = 5.02$, df = 3, 64; $P < 0.01$) in the four sizes of sand (Table 2). *Haustorius canadensis* preferred the finest sand (median diameter = 0.228 mm); *N. biarticulatus* selected sands with median diameters of 0.308 and 0.228 mm with equal frequency. The finest sand (0.228 mm) contained six times as much plant pigment as any of the other fractions. There

FIG. 2. Mortality rates of the five haustoriids in waters of different salinities: A, *Haustorius canadensis*; B, *Neohaustorius biarticulatus*; C, *Acanthohaustorius millsi*; D, *Parahaustorius longimerus*; E (*top, opposite page*), *Protohaustorius deichmannae*. Experiment with *A. millsi* carried out at 20 C and others at 15 C.

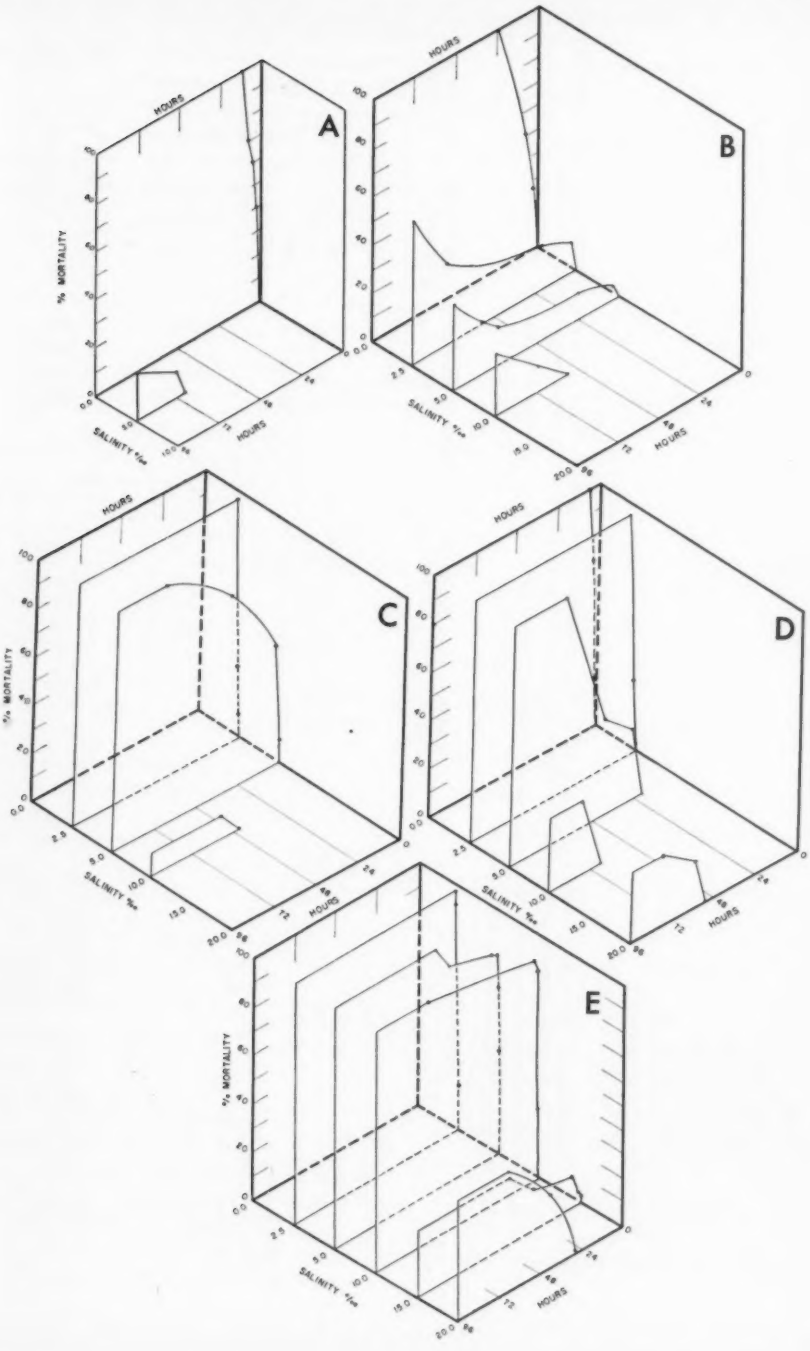
A
B
C
D
E
% MORTALITY
HOURS
SALINITY ‰

TABLE 1. Percentages of the various haustoriids found, 48 hr after release, in various sizes of sand that was screened while wet. Values joined by vertical lines are not significantly different at $P = 0.05$, as tested by Duncan's new multiple range test.

Size range of sand (diam in *mm*)							
Percentile			*Haustorius canadensis*	*Neohaustorius biarticulatus*	*Acanthohaustorius millsi*	*Protohaustorius deichmannae*	*Parahaustorius longimerus*
90	50	10					
2.00	1.50	1.00	2.9	1.5	2.3	0	0
0.930	0.817	0.530	38.3	45.0	18.4	9.6	31.6
0.500	0.360	0.330	40.1	45.0	40.5	47.6	40.0
0.372	0.283	0.210	18.5	8.4	38.8	42.8	28.4
No. specimens			167	131	304	250	60
No. replicates			10	9	12	10	3

TABLE 2. Percentages of *Haustorius canadensis* and *Neohaustorius biarticulatus* found 48 hr after release in sands subjected to various treatments.

Treatment of sand				*H. canadensis*	*N. biarticulatus*
Sand dried at 40 C					
Diam of sand (*mm*)					
Percentile			%		
90	50	10	organic matter		
1.100	0.540	0.425	0.220	21 (169, 22)[a]	23 (365, 17)
0.425	0.375	0.295	0.117	21	13
0.341	0.308	0.245	0.105	17	31
0.299	0.228	0.175	0.223	41	33
Sand ashed at 500 C					
Diam of sand (*mm*)					
Percentile					
90	50	10			
1.100	0.540	0.425		10 (130, 15)	22 (251, 10)
0.425	0.375	0.295		35	26
0.341	0.308	0.245		35	31
0.299	0.228	0.175		19	21
Untreated sand				84 (130, 12)	64 (389, 12)
Sand dried at 40 C				16	36
Untreated sand				72 (105, 9)	
Sand dried at 40 C and then soaked in sea water for 5 days				28	
Untreated sand				62 (158, 16)	63 (267, 11)
Sand ashed at 500 C				38	37
Sand dried at 40 C				54 (223, 23)	59 (170, 10)
Sand ashed at 500 C				46	41
Untreated sand from Sippewissett Creek (mean grain size = 0.364 mm)				48 (137, 15)	37 (400, 10)
Untreated sand from Nobska Beach (mean grain size = 0.256 mm)				52	63
Sippewissett sand ashed at 500 C				46 (92, 9)	49 (96, 7)
Nobska sand ashed at 500 C				54	51
Untreated Sippewissett sand (mean grain size = 0.364 mm)				90 (83, 10)	71 (377, 9)
Untreated Barnstable Harbor sand (mean grain size = 0.271 mm)				10	29
Sippewissett sand ashed at 500 C				54 (190, 20)	55 (343, 8)
Barnstable Harbor sand ashed at 500 C				46	45

[a]Numbers of specimens and replicates.

was no noticeable difference in the amount of pigment between the other fractions of sand. This was expected, since the small, single-celled algae worked their way through the screens and were concentrated in the last and finest fraction. The coarsest and the finest fractions had similar amounts of organic matter in them, about twice as great as that found in the other two fractions (Table 2). The organic matter in the coarsest fraction consisted of pieces of decaying cord grass, *Spartina* sp., from 0.5 to 1.0 mm in size. The organic material in the finest fraction was made up of diatoms and small pieces of cord grass (>0.3 mm).

When the species were given a choice of the four sizes of the above-mentioned sands after the organic matter was removed from the sands by ignition, the species preferred different sizes of sand than they did when the organic matter was still in the sand (Table 2). *Neohaustorius biarticulatus* was randomly found in the various lots of sand, and higher numbers of *H. canadensis* were found in sands with median grain sizes of 0.308 and 0.375 mm than in the fractions with sizes 0.540 or 0.228 mm ($F = 6.61$, df = 3, 56; $P < 0.01$).

Higher numbers ($P < 0.01$) of *H. canadensis* and *N. biarticulatus* were found in the untreated sand than in the same sand that had been dried at 40 C. This preference for the untreated sand was still shown by *H. canadensis* even after the dried sand had been soaked in filtered sea water for 5 days. No interstitial fauna were observed in the treated sand after 5 days, but bacteria were probably introduced from the untreated sand in the opposite compartments of the petri dish and the unfiltered sea water during the 48 hr of the experiment.

Both species preferred untreated sand to sand that had all its organic matter removed by ignition (*H. canadensis*, $t = 2.40$, df = 10, $P < 0.05$; *N. biarticulatus*, $t = 2.52$, df = 10, $P < 0.05$). But when given the alternative between ignited sand and sand that was dried at 40 C neither species showed a significant preference.

Neohaustorius biarticulatus preferred sands from Nobska over sands from Sippewissett Creek ($t = 3.89$; df = 10; $P < 0.05$); *H. canadensis* showed no preference. When the organic matter was removed from the two types of sand neither species showed a preference for either sand.

The experiment with untreated Barnstable Harbor and Sippewissett sands showed that the species preferred ($P < 0.01$) the Sippewissett sand. When the organic matter was removed from the two sands the species did not show a preference for either sand. The untreated Barnstable Harbor sand had a darker colour and a stronger smell than the Sippewissett sand. Barnstable Harbor sand tended to become anaerobic after 4 or 5 days when held in a container with sea water. This did not happen with Sippewissett Creek sand.

Rates of Oxygen Consumption and Low Oxygen Tolerances

An analysis of variance showed that the rates of oxygen consumption differed among the species ($F = 17.90$; df = 4, 22; $P < 0.01$) (Table 3).

TABLE 3. Mean oxygen consumption rates and concentrations of oxygen at which 50% of the specimens of each species died.

Species	Mean oxygen consumption rate[a]	Mean 50% lethal oxygen concentration[a]	No. replicates
Haustorius canadensis	204±39[b]	336±27*[c]	4
Acanthohaustorius millsi	430±12*	261±41**	5
Parahaustorius longimerus	459±176*	332±58*,**	5
Neohaustorius biarticulatus	776±37**	375±53*	5
Protohaustorius deichmannae	786±184**	397±173	9

[a]Oxygen consumption rate expressed as μliter/hr per g dry weight and lethal oxygen concentration as μliter/liter.

[b]One standard deviation of the mean of the sample.

[c]Values with equal numbers of asterisks(*) are not significantly different at $P < 0.05$.

Duncan's new multiple range test showed that *H. canadensis* had the lowest consumption rate and that *A. millsi* and *P. longimerus* had lower rates than *N. biarticulatus* and *P. deichmannae*.

Unpaired Student's *t*-tests showed that *A. millsi* had a lower lethal level than all species except *P. longimerus* and that *P. deichmannae* had a higher lethal level than all the other species (Table 3).

GILL AREA RELATED TO BODY WEIGHT, RATE OF OXYGEN CONSUMPTION, AND LOW LETHAL OXYGEN CONCENTRATION

The relation between gill area and body weight of the animals was linear for all species but *N. biarticulatus* (Fig. 3). As the *N. biarticulatus* data deviated from linearity ($F = 5.48$; df = 2, 11; $P < 0.01$), they were analysed in two segments, for weights from 1 to 2 mg and for those over 2 mg, to give two linear regressions. The regression for larger *N. biarticulatus* shows that this species had very little increase in the gill area with body weights over 2 mg. This was because the heavy animals were females with ripe ovaries; therefore, they increased in weight but not in overall size; Because of this, values for specimens over 2 mg were ignored in the covariance calculations.

The regression coefficients and the adjusted means for all species were compared by an analysis of covariance (Li, 1957) and were not found different at $P < 0.05$.

No significant correlation was found between the gill area per unit weight of the different species and their rates of oxygen consumption or their low lethal oxygen levels.

DISCUSSION

The intertidal species, *H. canadensis* and *N. biarticulatus*, showed greater tolerances to high temperature, desiccation, and low salinities and preferred coarser sands than the subtidal species, *A. millsi*, *P. longimerus*, and *P. deichmannae*.

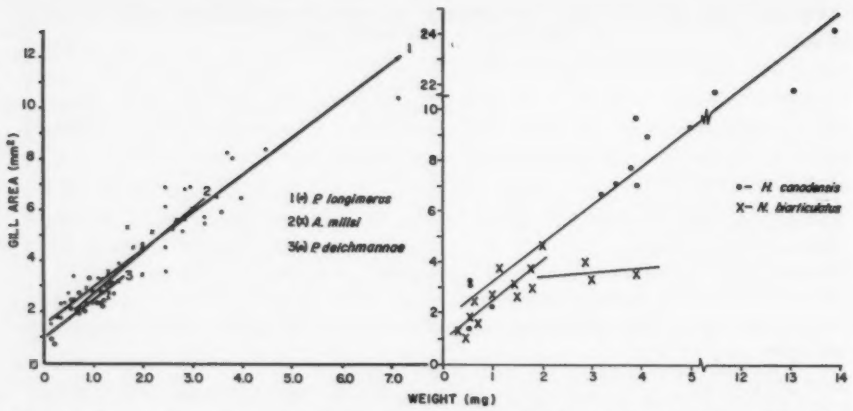

FIG. 3. Regression lines for gill area versus dry body weight for the five haustoriids. The equations for the various species were

Haustorius canadensis	Y = 1.62 + 1.75 X
Neohaustorius biarticulatus	
1–2 mg	Y = 0.99 + 1.41 X
2–4 mg	Y = 3.00 + 0.17 X
Acanthohaustorius millsi	Y = 1.04 + 1.64 X
Parahaustorius longimerus	Y = 1.08 + 1.49 X
Protohaustorius deichmannae	Y = 1.41 + 1.56 X

The greater tolerance to high temperature shown by *H. canadensis* and *N. biarticulatus* is in keeping with the fact that, being intertidal species, they are exposed to higher environmental temperatures (Sameoto, 1969a, b). Croker (1967) found that 100% of *P. longimerus* taken at Sapelo Island, Georgia, died in 15 min at 37 C. This indicates that the upper lethal temperature of this species is not appreciably higher in the warmer southern waters than at Cape Cod. Sprague (1963) stated that the 24-hr 50% lethal level for species of freshwater amphipods was much higher than the species' environmental temperature and, therefore, there was a considerable safety margin for direct lethal effects of temperature. Such a margin of safety existed with the species at Barnstable Harbor, where the maximum recorded temperature of the sand containing Haustoriidae was 27 C (Sameoto, 1969b), and at Sippewissett Creek, where the maximum environmental temperature was 31 C (Sameoto, 1969a).

The ability of a haustoriid to survive for prolonged periods of time in the humid interstitial air of intertidal sands above the water table is important if it is to occupy this zone successfully, since the animal may frequently be trapped in sands that are not covered by the next high tide. Croker (1967) found the minimum relative humidity of the interstitial air was always greater than 80%. Lagerspetz (1963) found the 50% survival time at 19C in 100% relative humidity for *Gammarus duebeni* Lilljeborg to be 172 hr; that for *Gammarus oceanicus* Segerstrale, 48 hr; and that for *Pontoporeia affinis* Lindstrom, 40 hr. *Gammarus duebeni* is adapted for short periods of terrestrial

life; the other two species are entirely aquatic. Williamson (1951) reported the survival time at 100% relative humidity and 25 C for the semiterrestrial amphipods *Talitrus saltator* (Montagu) and *Orchestia gammarella* (Pallas) to be greater than 48 hr. The 50% survival times of *H. canadensis* and *N. biarticulatus* are close to those of *G. duebeni* and *O. gamarella*; those for the other three species are less than those for *G. oceanicus* and *P. affinis*. The differences in the survival abilities of the species cannot be due to differences in body size, for *H. canadensis* and *P. longimerus* are similar in size, as are *A. millsi* and *N. biarticulatus*, but not in survival ability This suggests there has been an adaptation to withstand drying conditions in the case of *H. canadensis* and *N. biarticulatus*, although no adaptation has occurred in the other three species, since they did not survive so well as *G. oceanicus* and *P. affinis*.

These five species of haustoriids are not well adapted to withstand low salinities compared with amphipods recognized as brackish water species. Kinne (1959) found that *G. duebeni* tolerated salinities from freshwater to 73‰. *Corophium volutator*, an intertidal brackish water species, was found living in water of less than 2% (Segerstrale, 1959). Sanders et al. (1965) reported *Leptocheirus plumulosus* Shoemaker living in a salinity range of 3–31‰. *Gammarus oceanicus* tolerates salinities as low as 2–3‰ (Segerstrale, 1947) and 1‰ (Steen, 1951). Compared with these species the Haustoriidae are relatively stenohaline. This is supported by the findings of Vader (1965), who found *Haustorius arenarius* common in water less than 18.1‰ but preferring salinities greater than 29.8‰.

Of all the environmental factors to which benthic animals are exposed, one of the most important is the nature of the bottom sediments. It has been demonstrated many times for various groups of invertebrates that the animals respond to grain size and the quality and quantity of the organic matter in the sediments (Scheltema, 1961; Meadows, 1964; Crisp, 1965; Grey, 1966, 1967). Each haustoriid species of this study showed a preference for sands of a certain size range if the sands were not altered in any way before screening. The intertidal species preferred coarser sands than the subtidal species. This was expected since the intertidal species came from a region of coarser sands than the subtidal species (Sameoto, 1969a, b). When given a choice of different sizes of sand that had been dried before screening, *H. canadensis* and *N. biarticulatus* preferred finer sands than they did when choosing from sands that were screened while still wet. This meant that the quality or quantity, or both, of the organic matter in the sand affected the sediment preference. This was more clearly demonstrated by the preference shown by both species for untreated sands over sands that had their organic material altered by drying at 40 C. The factor the animals responded to may have been the absence of certain living flora and fauna found in the natural sands (Meadows and Anderson, 1968). This was supported by the results of the experiment giving the animals a choice between sand that had its organic matter removed by ashing at 500 C and sand that had all interstitial life killed, but no organic material removed, by drying at 40 C. The two species

showed no significant difference in their preference for these two sands. Meadows (1964) found that *Corophium volutator* (Pallas) and *C. arenarium* Crawford were sensitive to changes in the primary organic film of the sand and its constituent microorganisms and would reject sands in which these were changed or removed.

The experiments that provided a choice between sand from Sippewissett Creek and sand from Nobska Beach indicated that the sediment was not the factor that prevented *N. biarticulatus* and *H. canadensis* from inhabiting Nobska Beach. The experiments with Barnstable Harbor sand showed, however, that the avoidance of this sand by the two species was related to either the low oxygen conditions of the sand or the organic material in the sand, or both.

Why the five species preferred the different sizes of sand sorted when wet is open to conjecture, but the explanation may be related to the body size and shape of the animals. Since the five species appeared to burrow alike (Sameoto, 1969a), by passing the sand grains between the peraeopods, it is possible that they have developed a preference for sand that contains a major portion of the grains small enough to pass through the space between their peraeopods. This may partially explain why *P. deichmannae*, the species with the narrowest space between its peraeopods, prefers the finest sands. It is more difficult to explain the difference in sand size preference between *N. biarticulatus* and *A. millsi* for they are approximately the same size. *Acanthohaustorius millsi* may have selected the finer sands because of its preference for the subtidal areas. The coarsest sands are generally found higher on the beach (Hedgpeth, 1957); therefore, if *A. millsi* sought fine sands, their chances of being trapped intertidally at low tide would be less than if they selected coarser sands similar to those chosen by *N. biarticulatus*. The reason for the difference may also simply be that *A. millsi* has feeding habits that are more efficient in finer sands.

All experiments on the sediments were carried out with only adult animals. The response of the newly released juveniles may be very important in determining the distribution of the species. The young must be able to burrow into the sand on release from the brood pouch (Sameoto, 1969a). If the sands were too coarse for the young to dig in, but not too coarse for the adults, this could result in disaster for the new generation, for they would likely be exposed to heavy predation.

Acanthohaustorius millsi and *P. longimerus* showed greater tolerance to low oxygen concentrations than other species tested. These two species were found in sands at Nobska Beach and Barnstable Harbor that showed signs of being anaerobic 2 or 3 cm below the surface (Sameoto, 1969b).

Acanthohaustorius millsi was the only species of Haustoriidae found in the subtidal sands of Black Beach (Sameoto, 1969a). These sands also showed anaerobic conditions about 2 cm below the surface and this may be one reason why the intertidal species *H. canadensis* and *N. biarticulatus* are not found in them. *Protohaustorius deichmannae* had less tolerance for low oxygen concentrations than *A. millsi* and *P. longimerus*. It was also found at Nobska

Beach and Barnstable Harbor, but occupied the upper layers of sand, which probably have a better supply of oxygen than the lower sands, occupied by the other two species.

A better indication of the required oxygen conditions of the animals than the tolerance for low oxygen concentrations may be shown by the oxygen consumption rates. *Haustorius canadensis* had a rate lower than the other four species, whereas the other species fell into two groups. The species with the highest oxygen consumption rates, *N. biarticulatus* and *P. deichmannae*, are the ones that occupy the uppermost layers of sand (Croker, 1967; Sameoto, 1969a, b). *Haustorius canadensis* is found in deeper, less oxygenated sands (Brafield, 1964) than *N. biarticulatus*, the species it is associated with.

Exposure to high temperature and interstitial air in the sands above the water table may be the factors that prevented *A. millsi*, *P. longimerus*, and *P. deichmannae* from occupying sands in the upper regions of the intertidal zone. At Barnstable Harbor these species are found in intertidal sands that remain water saturated and do not exceed 27 C at low tide (Sameoto, 1969b). Bousfield (1965) and Dexter (1967) reported *A. millsi* and *P. longimerus* inhabited wet sands of the lower intertidal zone and subtidal zone, whereas *P. deichmannae* was found mainly in subtidal sands.

Of these three species only *A. millsi* is found at Black Beach and it was seldom present intertidally even in water-saturated sands. The intertidal sands are coarser (mean range = 0.455–0.365 mm) than in the subtidal area (mean = 0.271 mm) (Sameoto, 1969a). Therefore, since *A. millsi* showed preference for the finest sands offered in the experiments, it seems probable that sediment size played a role in its habitat selection.

Haustorius canadensis and *N. biarticulatus* had the same upper lethal temperature, but *H. canadensis* was able to withstand desiccation longer than *N. biarticulatus*. Yet it was *N. biarticulatus* that was found in the warmest and driest sands in the intertidal zone; *H. canadensis* was most common in the sands that remained water saturated (Sameoto, 1969a). Why *H. canadensis* was not found more frequently in the upper intertidal sands is not known, as the experiments showed it was capable of withstanding the environment. Neither intertidal species was found in the subtidal regions of Black Beach, probably because of the finer sands in the subtidal area, for experiments with sand taken from this area showed the species preferred the coarser fractions of sand. The anaerobic conditions of the subtidal area may also have influenced the intertidal species more than *A. millsi*, for both had lethal oxygen concentrations higher than those of *A. millsi*.

REFERENCES

BOUSFIELD, E. L. 1965. Haustoriidae of New England (Crustacea: Amphipoda) Proc. U.S. Natl. Museum 117: 159–239.

BRAFIELD, A. E. 1964. The oxygen content of interstitial water in sandy shores. J. Animal Ecol. 33: 97–116.

CONOVER, R. J. 1956. Oceanography of Long Island Sound 1952–54. VI. Biology of *Acartia clausi* and *A. tonsa*, p. 156–233. *In* Oceanography of Long Island Sound. X. Bingham Oceanog. Collection 15.

1960. The feeding behavior and respiration of some marine planktonic Crustacea. Biol. Bull. 119: 399–415.

CRISP, D. J. 1965. Surface chemistry, a factor in the settlement of marine invertebrate larvae. Botan. Gothoburgensia 3: 51–65.

CROKER, R. A. 1967. Niche diversity in five species of intertidal amphipods (Crustacea: Haustoriidae). Ecol. Monographs 37: 173–200.

DEXTER, DEBORAH M. 1967. Distribution and niche diversity of haustoriid amphipods in North Carolina. Chesapeake Sci. 8: 187–192.

FRY, F. E. J., J. R. BRETT, AND G. H. CLAWSON. 1942. Lethal limits of temperature for young goldfish. Rev. Canadienne Biol. 1: 50–56.

GRAY, J. S. 1966. The attractive factor of intertidal sand to *Protodrilus symbioticus*. J. Marine Biol. Assoc. U.K. 46: 627–645.

1967. Substrate selection by the archiannelid *Protodrilus rubropharyngeus*. Helgolaender Wiss. Meeresuntersuch. 15: 253–269.

HEDGPETH, J. W. 1957. Sandy beaches, p. 587–608. *In* J. W. Hedgpeth, [ed.] Treatises on marine ecology and paleoecology. Geol. Soc. Am. Mem. 67: 1.

KINNE, O. 1959. Ecological data on the amphipod *Gammarus duebeni*. A monograph. Veroeffentl. Inst. Meeresforsch. Bremerhaven 6: 177–202.

LAGERSPETZ, KARI. 1963. Humidity reactions of three aquatic amphipods, *Gammarus duebeni*, *G. oceanicus* and *Pontoporeia affinis* in the air. J. Exptl. Biol. 40: 105–110.

LI, J. C. R. 1957. Introduction to statistical inference. Edwards Brothers Inc., Ann Arbor, Michigan. p. 568.

MEADOWS, P. S. 1964. Experiments on substrate selection by *Corophium* species; films and bacteria on sand particles. J. Exptl. Biol. 41: 499–512.

MEADOWS, P. S., AND J. G. ANDERSON. 1968. Micro-organisms attached to marine sand grains. J. Marine Biol. Assoc. U.K. 48: 161–175.

MCLEESE, D. W. J. 1956. Effect of temperature, salinity and oxygen on survival of lobsters. J. Fish. Res. Bd. Canada 13: 247–272.

SAMEOTO, D. D. MS, 1967. The comparative ecology of five species of sand-burrowing intertidal amphipods (Crustacea: Haustoriidae). Ph.D. Thesis. Queen's University, Kingston, Ont. 239 p.

1969a. Comparative ecology, life histories, and behaviour of intertidal sand-burrowing amphipods (Crustacea: Haustoriidae) at Cape Cod. J. Fish. Res. Bd. Canada 26: 361–388.

1969b. Some aspects of the ecology and life cycle of three species of subtidal sand-burrowing amphipods (Crustacea: Haustoriidae). J. Fish. Res. Bd. Canada 26: 1321–1345.

SANDERS, H. L., P. C. MANGELSDORF, JR., AND G. R. HAMPSON. 1965. Salinity and faunal distribution in Pocasset River, Massachusetts. Limnol. Oceanog. Suppl. 10: R216–R229.

SCHELTEMA, R. S. 1961. Metamorphosis of the veliger larvae of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. Biol. Bull. 120: 92–109.

SEGERSTRALE, S. G. 1947. New observations on the distribution and morphology of the amphipod *Gammarus zaddachi* Sexton with notes on related species. J. Marine Biol. Assoc. U.K. 27: 219–244.

1951. Synopsis of data on the crustaceans *Gammarus locusta*, *G. oceanicus*, *Pontoporeia affinis* and *Corophium volutator* (Amphipoda: Gammaridae). Soc. Sci. Fennica Commentationes Biol. 20: 1–23.

SPRAGUE, J. B. 1963. Resistance of four freshwater crustaceans to lethal high temperature and low oxygen. J. Fish. Res. Bd. Canada 20: 386–415.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Inc., New York. p. 481.

STEEN, E. 1951. Ecological observations on some *Gammarus* and *Marinogammarus* species on the Scandinavian west coast. Oikos 3: 232–242.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1965. A manual of sea water analysis. Bull. Fish. Res. Bd. Canada 125. p. 123.

THOMAS, H. J. 1954. The oxygen uptake of the lobster (*Homarus vulgaris*). J. Exptl. Biol. 31: 228–251.

VADER, W. J. M. 1965. Intertidal distribution of haustoriid amphipods in the Netherlands. Botan. Gothoburgensia 3: 233–246.

WILLIAMSON, E. I. 1951. Studies in the biology of Talitridae (Crustacea: Amphipoda). Effects of atmospheric humidity. J. Marine Biol. Assoc. U.K. 30: 73–90.

ZEIGLER, J. M., G. G. WHITNEY, JR., AND C. R. HAYES. 1960. Woods Hole sediment analyser. J. Sediment. Petrol. 30: 490–495.

Effect of Hematin Compounds on the Development of Rancidity in Muscle of Cod, Flounder, Scallops, and Lobster[1]

C. H. CASTELL AND D. M. BISHOP

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

CASTELL, C. H., AND D. M. BISHOP. 1969. Effect of hematin compounds on the development of rancidity in muscle of cod, flounder, scallops, and lobster. J. Fish. Res. Bd. Canada 26: 2299–2309.

Hemoglobin, catalase, myoglobin, and inorganic iron were added to blended muscle from cod, flounder, scallops, and lobsters to determine the effects of these various forms of iron on the oxidation of the muscle lipids as indicated by increased malonaldehyde and rancid odours. Hemoglobin and catalase were very active catalysts; myoglobin was relatively inert. Scallop muscle was slightly more susceptible to hemoglobin-induced rancidity than muscle from cod or flounder; lobster muscle was highly resistant. There was a difference in the odours accompanying the development of rancidity after the addition of free Fe^{++} and hemoglobin-bound Fe^{++}. An increase in the free fatty acid content of the muscle retarded the hemoglobin-induced rancidity. Certain free amino acids, notably tryptophane and proline, also had an inhibitory action, whereas other amino acids had a prooxidant effect.

Received February 4, 1969.

INTRODUCTION

A PREVIOUS PAPER (Castell and Spears, 1968) showed differences between species of fish and between other marine animals in susceptibility of muscle to rancidity induced by the addition of heavy metal ions. This prompted the question whether species of fish differ in susceptibility of muscle to rancidity induced by the addition of hematin compounds.

In this paper an account is given of the effect of adding various concentrations of hemoglobin, myoglobin, and catalase to muscle from cod, flounder, scallops, and lobster. The results showed that these organic iron catalysts, like the inorganic iron catalysts, differ in effectiveness in inducing rancidity, both within and between species.

Some related experiments were also carried out: Fe^{++}, Fe^{+++}, Cu^{++}, and hemoglobin iron were compared for effectiveness for inducing rancidity in the fish muscles, and free amino acids, and other compounds, for effects on the development of hemoglobin-induced rancidity.

In certain marine animals there is a point of additional interest: some species of mollusks and arthropods are characterized by having the copper-containing hemocyanin as the oxygen carrier in their blood in place of hemoglobin. Tests were carried out to determine whether muscle from one of these

[1]The 11th paper in a series dealing with rancidity in fish muscle.

Printed in Canada (J1360)

hemoglobin-free arthropods was more or less sensitive to hemoglobin-induced rancidity than muscles from other marine species.

Although hematin compounds are essentially catalysts for cell respiration, it is now widely recognized that they are also the most important of the naturally occurring lipid oxidation catalysts in animal tissues (Watts, 1954; Tappel, 1953). Their role in lipid oxidation in general and in the development of rancidity in meats has been reviewed by Watts (1954), Tappel (1961, 1962), and Hoynak (MS, 1962). A brief summary of their importance in the oxidative deterioration of fishery products has been given by Tappel (1965).

The Natural Hematin Content of Fish Muscles

There is a greater range in the concentration of the hematin compounds in muscle from different species of fish than from different species of mammals. These compounds are present in relatively large concentrations in the muscle from most fatty fish, especially in the lateral band of dark muscle. It is the hemoglobin and myoglobin that give to this muscle in herring and mackerel their characteristic brownish colour. These catalysts are present in much smaller concentrations in the flesh of lean fish (Banks, 1967). According to Stansby and Brown (1958) cod and halibut have concentrations of $0.1\text{–}0.4 \times 10^{-5}$ M, whereas the more fatty pilchard and tuna have concentrations of 5.4×10^{-5} and 8.5×10^{-5} M respectively. These authors point out that, in a general way, fish with the highest hematin content are those that are most susceptible to oxidative rancidity. Other data dealing with the concentration of hematin compounds in fishery products are found in Matsuura and Hashimoto (1959), Nicol (1960), Brown (1962), Brown et al. (1962), and Altman and Dittmer (1964).

PROCEDURES AND MATERIALS

The hematin compounds were obtained from commercial sources: crystalline, lyophilized horse heart myoglobin, lyophilized catalase, twice recrystallized hemin, and hemoglobin, from Nutritional Biochemicals Incorp.; other samples of hemoglobin from Difco and from Matheson, Coleman and Bell. Calculations for the iron content of these compounds were based on the following approximate molecular weights: hemoglobin 68,000 — with 4 atoms of Fe; horse heart myoglobin 17,000 — with one atom of Fe; catalase 225,000 — with 4 atoms of Fe.

To minimize the effect of the normally present hematin compounds, four species were selected in which these organic iron compounds are known to be in low concentrations in the muscle. Fresh, high quality, cod (*Gadus morhua*) and flounder (*Hippoglossoides platessoides*) were specially selected from the last day's catch (24–48 hr in ice) from deep-sea trawlers. Lobsters (*Homarus americanus*) were obtained live from this laboratory's aquaria. Only the freshly excised tail meat of lobsters was used. The adductor muscles of scallops (*Placopecten magellanicus*) were shipped in ice from Lunenburg and were always from the last day's catch of the scallop draggers. Either whole fillets, or the specified whole muscles from the other animals, were prepared as a blend using one part of tissue to three parts of water. The preparation and storage of these blends have been described previously (MacLean and Castell, 1964). After the reagents were added to the blended muscle they were mixed by stirring or blending lightly and then stored in foil capped flasks at 0 C.

The extent of rancidity in the muscle was measured in terms of malonaldehyde, by the thiobarbituric acid reaction, using the procedure of Tarladgis et al. (1960). "Malonaldehyde

value" is the number of milligrams per 1000 g of muscle. To prevent oxidation during distillation, EDTA and propyl gallate were added to the flasks when free Fe^{++} or Fe^{+++} was known to be present (Castell et al., 1966b). This precaution was not necessary with hemoglobin, myoglobin, or catalase. Optical density readings were made with a Beckman DU Spectrophotometer at 538 mμ.

To make direct comparisons of their activity, the concentrations of the catalysts were recorded as parts per million (ppm) of the metal component added to the blended muscle.

TESTS WITH COD MUSCLE

Catalase and hemoglobin were both very active but the malonaldehyde values were always slightly higher for catalase than for hemoglobin (Fig. 1). Myoglobin, on the other hand, was relatively inert. Concentrations up to 16 ppm of myoglobin iron induced no rancid odours and only small increases in the malonaldehyde content. Hemin was even less active: up to 85 ppm of hemin iron had no observable effect on the development of rancidity in the cod muscle.

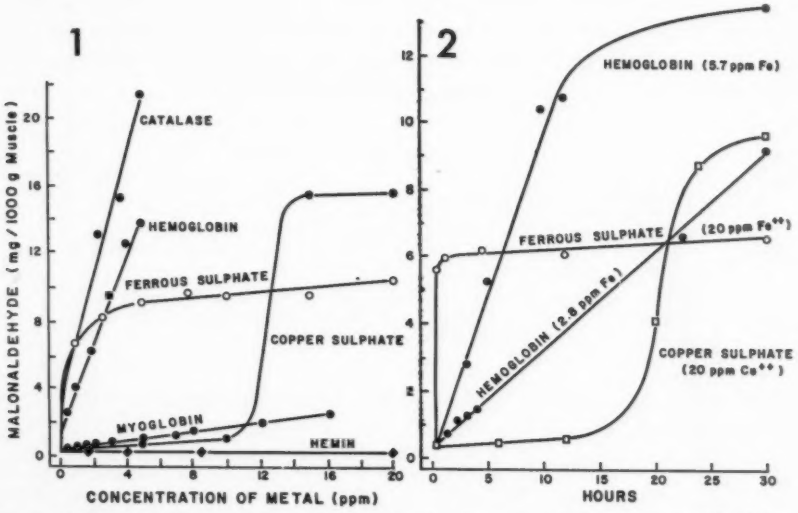

FIG. 1. Effect of various metal catalysts on the development of rancidity in blended cod muscle held for 24 hr at 0 C.

FIG. 2. Effect of time at 0 C on the development of rancidity in cod muscle after the addition of hemoglobin (2.8 and 5.7 ppm Fe) and 20 ppm Cu^{++} and Fe^{++} as inorganic salts.

The relation between the concentration of iron and the development of rancidity was different for the free and the bound ferrous ions. Below 1.5–2 ppm, free Fe^{++} induced more rancidity, and produced it more quickly, than corresponding amounts of bound iron in the form of hemoglobin. As the concentration of iron was increased above this level, the slope of the malonaldehyde curve for the free Fe^{++} leveled off whereas that for the hemoglobin and catalase continued upward to much higher values. Rancid and

associated off-odours developed almost immediately after the addition of of free Fe^{++} to the blended muscle; a much longer period was required for the development of hemoglobin-induced odours. The rate of malonaldehyde development varied correspondingly (Fig. 2, 3). However, compared with Cu^{++} the induction period for hemoglobin was relatively short (Fig. 2).

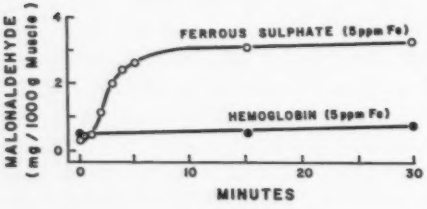

FIG. 3. Effect of short holding periods at 0 C on the development of rancidity in blended cod muscle after the addition of 5 ppm Fe as hemoglobin and as an inorganic iron salt.

Tests were made throughout the year to compare the effect of bound and free Fe^{++} on muscle cut from fish at various seasons. Although the values were not the same for all muscle samples, the general relationship shown in the examples in Fig. 4 was always obtained.

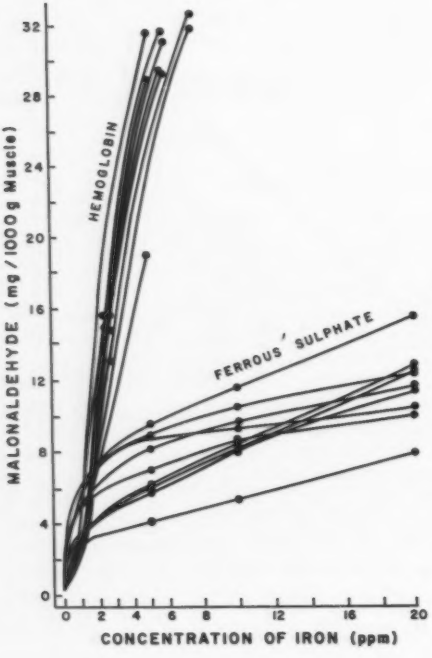

FIG. 4. Effect of iron, added as hemoglobin and as ferrous sulphate, on the development of rancidity in blended cod muscle held for 24 hr at 0 C for fish caught between February 5 and April 30, 1968.

Effect of Free Fatty Acids (FFA)

Increases in the FFA content of the muscle had an inhibiting action on the development of rancidity catalyzed by hemoglobin. This was observed under three different conditions. In the first test (Fig. 5), fish were held in frozen storage until their FFA content had increased from about 5% to values between 45 and 65%; in the second test (Fig. 6), a mixture of fatty acids,

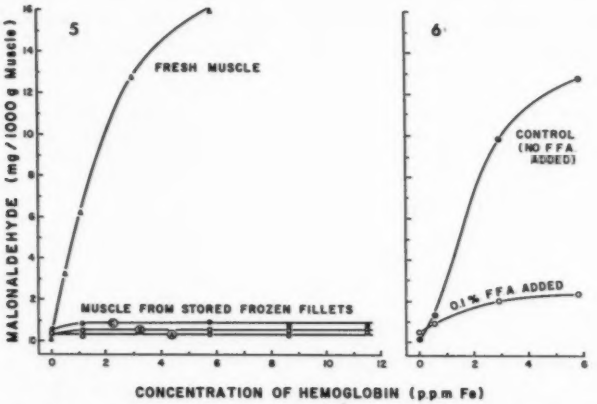

Fig. 5. Effect of hemoglobin on the development of rancidity in a blend prepared from a fresh cod fillet having a FFA content of 4.6% of the total lipid, compared with the effect of hemoglobin on blends prepared from old, stored, frozen fillets having FFA values of (A) 65%, (B) 57%, and (C) 48%.

Fig. 6. Effect of adding 0.1% FFA, obtained from cod-liver oil, on the development of hemoglobin catalyzed rancidity in blended cod muscle held at 0 C for 24 hr.

obtained from hydrolyzed cod-liver oil, was added to fresh muscle; and in the third test (Table 1) the FFA content of fresh cod muscle was increased by means of snake venom phospholipidase. These results were all similar to those

Table 1. Effect of free fatty acids, produced in cod muscle by the addition of phospholipidase-rich snake venom, on the susceptibility of the muscle to rancidity catalyzed by Cu++, Fe++, and hemoglobin. (Snake venom, 0.1%, was added to blended muscle and held for 24 hr at 0 C. Free fatty acids in the muscle increased from 7.7 to 66% of the total lipids. The catalysts were then added and left for another 24 hr at 0 C.)

Catalyst	Malonaldehyde values (*mg/1000 g muscle*)		% inhibition
	−Snake venom	+Snake venom	
Cu++, 20 ppm	9.14	0.463	94.9
Fe++, 20 ppm	8.29	2.24	72.9
Hemoglobin Fe, 2.88 ppm	7.76	0.712	90.8

found previously (Castell et al., 1966a, b) in which rancidity induced by Cu^{++} in cod muscle was retarded by increased concentrations of FFA.

Effect of Amino Acids

The addition of certain amino acids and protein hydrolysates also affected the rate of the production of hemoglobin-induced rancidity. Some amino acids, notably tryptophane and proline, inhibited the reaction whereas others, particularly cysteine and asparagine, increased the amount of rancidity; others had little or no effect (Table 2). Similar results were obtained when

Table 2. Effect of amino acids and yeast hydrolysate on hemoglobin-catalyzed rancidity in blended cod muscle during a period of 24 hr at 0 C. Concentrations used were: amino acids 0.04 M; yeast hydrolysate 0.5%; hemoglobin 1.5 ppm.

Amino acid	Malonaldehyde value	% change	Activity
L-cysteine	11.65	+186.9	Prooxidant
DL-asparagine	8.22	+102.4	Prooxidant
DL-methionine	4.40	+8.8	?
DL-phenylalanine	4.28	+4.6	?
Control (No added amino acid)	4.06	–	–
L-leucine	3.88	−4.4	?
Hydroxy-L-proline	3.46	−14.7	Antioxidant
Glycyl-L-tyrosine	3.40	−16.2	"
Glycine	2.68	−33.9	"
L-proline	1.78	−56.1	"
DL-tryptophane	1.47	−61.3	"
Yeast hydrolysate	2.77	−31.8	"

these same amino acids were added to cod muscle in which rancidity was induced by the addition of free Fe^{++}. With the Fe^{++}, however, the amino acids were effective in lower concentrations in relation to the amount of iron that was added. The results with cysteine were of particular interest: in low concentrations the cysteine had a strong prooxidant action, but when the concentration was increased beyond a certain level (in relation to the amount of iron) it then became an antioxidant. When Cu^{++} was used in place of Fe^{++} or hemoglobin as the oxidation catalyst, the amino acids were effective in still smaller concentrations.

In preliminary experiments dealing with the effect of amino acids on hemoglobin-induced rancidity the results were often indefinite and confusing. It was later learned that this was due to using too high a concentration of hemoglobin in relation to the amount of amino acid. For example: when 0.025 M concentrations of amino acids were added to blended muscle to which 5 ppm hemoglobin iron was also added, the antioxidant effect of tryptophane or proline was negligible; when the hemoglobin iron was reduced to 1.5 ppm

and the amino acids increased to 0.05 M concentration the antioxidant action was evident and the results obtained were reproducible.

TESTS WITH MUSCLE FROM OTHER SPECIES

Flounder and scallop muscle were similar to cod muscle in being very sensitive to added hemoglobin (Fig. 8). Where direct comparisons were made

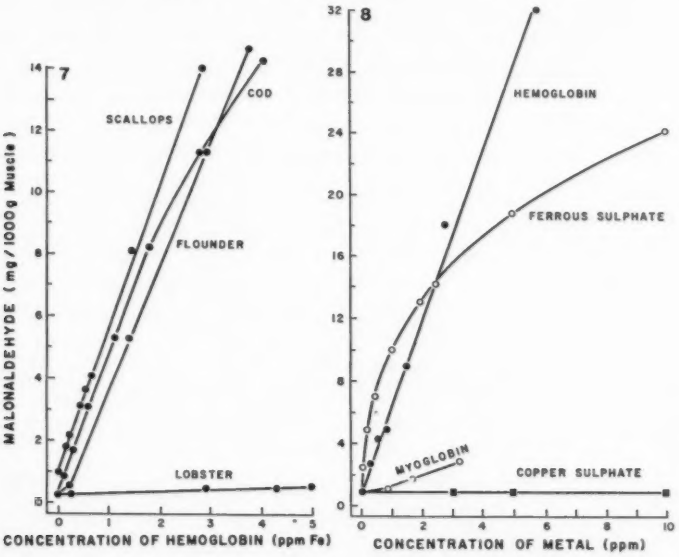

FIG. 7. Effect of hemoglobin on the development of rancidity in blended muscle from scallops, flounders, cod, and lobster after 24 hr at 0 C.

FIG. 8. Effect of hemoglobin, myoglobin, ferrous sulphate, and copper sulphate on the development of rancidity in blended scallop muscle held at 0 C for 24 hr.

the malonaldehyde values of the scallop muscle were always slightly higher than those of any other species that was tested. The scallop muscle was also slightly more sensitive than muscle from cod or flounder to added myoglobin, although this compound was still very much less active than the hemoglobin. As found previously (Castell and Spears, 1968) the scallop muscle was relatively insensitive to Cu^{++}, but much more sensitive to free Fe^{++} or Fe^{+++} (Fig. 7).

The lobster muscle differed from cod and flounder in being highly resistant to rancidity catalyzed by the addition of hemoglobin (Fig. 9). Hemoglobin iron (added to give 10–12 ppm) brought about no observable change; 40–50 ppm produced only small increases in malonaldehyde and very slight off-odours, but never typical rancid odours. The lobster muscle was also very resistant to rancidity induced by the addition of free Fe^{++} or Cu^{++}.

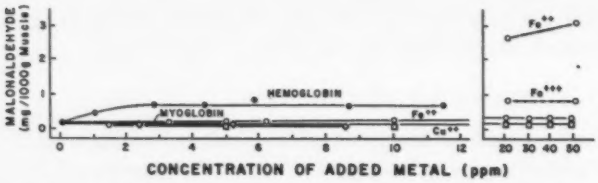

FIG. 9. Effect of the concentration of iron as hemoglobin, myoglobin, and ferrous sulphate, and of copper as copper sulphate, on the development of rancidity in lobster (tail) muscle, as shown by malonaldehyde values after 24 hr at 0 C. The values in the broken lines (*right*) are from an earlier experiment in which ferric sulphate and ferrous sulphate were added to lobster muscle.

ODOURS ASSOCIATED WITH HEMOGLOBIN-INDUCED RANCIDITY

In this and in preceding studies on metal-induced rancidity (Castell et al., 1965, 1966a; Castell and MacLean, 1966; Castell and Spears, 1968) it was observed that lipid oxidation in fish tissues was accompanied by a successive series of odours, only those in the final stages being characteristic of rancid oil. The variation of odours in the series and their intensity differed with muscle from different species and with different oxidation catalysts. Increased rancidity judged organoleptically is usually accompanied by increased malonaldehyde content; but this relationship is also subject to wide variations, again depending upon the nature of the catalyst and differences in the substrate (Castell et al., 1965, 1966a; Castell and Spears, 1968).

With these considerations in mind the following is a brief summary of our experience with odours accompanying hemoglobin-induced rancidity in cod muscle. The intensity of rancid and other off-odours is less with hemoglobin than it is with Cu^{++} or Fe^{++}. The initial "metallic," "salt-fish," and "seaweedy" odours, which are pronounced with Cu^{++}, and somewhat less with Fe^{++}, are almost absent with hemoglobin. The most pronounced and the most characteristic odours with hemoglobin are the final "oily-rancid" stage. It was frequently observed that malonaldehyde values of 3–6, which with Cu^{++} or Fe^{++} would be accompanied by strong "seaweedy-rancid" odours, had only "slight off-odour" with hemoglobin.

SUMMARY AND DISCUSSION

Hemoglobin and catalase were extremely active catalysts of lipid oxidation when added to blended muscle tissue from cod, flounder, and scallops. Rancid odours and high malonaldehyde values developed in less than 24 hr at 0 C after the addition of 0.5–5 ppm catalase or hemoglobin-bound iron. Although the active metal in both these hematin compounds is ferrous iron, its effect on the development of rancidity differed in several ways from that produced by adding free Fe^{++} in the form of a soluble iron salt. After the addition of these hematin compounds there was much less development of the initial "metallic," "salt-fish," or "seaweedy" odours that are observed during this

early stage when free Fe^{++} has been added. Instead, the most pronounced odours associated with hemoglobin-induced rancidity were the "fish-oil" and "oily-rancid" types that are usually associated with oxidizing marine oils. There were also characteristic differences in the rate and in the amount of malonaldehyde formed in these fish tissues after the addition of comparable amounts of the bound and free Fe^{++}. The free Fe^{++} reacted very quickly producing off-odours and increased malonaldehyde values within a few minutes. The malonaldehyde formation after the addition of hemoglobin was slower, but reached maximum values usually two to three times those produced by the free Fe^{++}. Because of these differences, a scale of rancidity, based on the relationship between odours and malonaldehyde values for rancidity induced by Fe^{++}, is of little or no value when applied to rancidity induced by the addition of hemoglobin or catalase.

Free fatty acids in the fish muscle retarded hemoglobin-induced rancidity. This was observed in fish where the FFA had increased during prolonged frozen storage; in other fish where the increased FFA had been brought about in less than 24 hr through the activity of added phospholipidase; and in fresh muscle to which small quantities of hydrolyzed marine oils had been added.

Free amino acids differed in their effect on hemoglobin-induced rancidity. Tryptophane and proline were antioxidant; cysteine and asparagine were prooxidant; others, including leucine, phenylalanine, and methionine had relatively little effect. It was also observed that for a given concentration of amino acids the effect was greater on free Fe^{++} than on hemoglobin-bound Fe^{++}.

Unlike the hemoglobin and catalase, the addition of corresponding concentrations of horse heart myoglobin had little or no effect on the development of rancidity in the fish muscles. This was unexpected, as others (Brown et al., 1963; Tappel, 1961) have found myoglobins to be active lipid oxidation catalysts. However, myoglobins from different species of animals are not identical (Brown et al., 1962), and it could be that if myoglobin from marine fish or mammals had been available, the results would have been different. In this connection an experienced worker in this field (Brown, 1962) has made this comment: "There has been a tendency in the past to assume that the properties of myoglobin and hemoglobin were sufficiently similar to make it possible to apply any results obtained with either of these proteins to any color problem or question concerning fish and meats. It can be seen that such assumptions are misleading." In the concentrations used, the hemin had no catalytic action. This chloride of ferric porphyrin is insoluble in water and it was found previously (MacLean and Castell, 1964) that insoluble salts of Fe^{+++}, Fe^{++}, or Cu^{++} had no effect on fish muscle lipids when added in the concentrations used in these experiments.

The muscle from the four species tested was not equally susceptible to hematin-induced rancidity. The scallops were always the most susceptible, the cod and flounders were somewhat less susceptible, and the lobster was extremely resistant. The present and previous observations (Castell and Spears,

1968) have shown that scallop muscle is also very sensitive to rancidity induced by Fe^{++} and Fe^{+++} when added as inorganic salts.

Watts (1961) found that crab meat was resistant to rancidity and Olcott (1962) suggests that these and other crustaceans may be stable because of their absence of myoglobin and hemoglobin. The present results suggest that this is not the correct explanation. Even when these compounds are added in relatively large concentrations they do not induce rancidity. Castell and Spears (1968) found that muscle from lobster, shrimp, and several species of crabs showed the same resistance to rancidity induced by the addition of Fe^{+++}, Fe^{++}, Cu^{++}, Co^{++}, and several other heavy metal ions. They further observed that muscle from cod could be protected against metal-induced rancidity by the addition of lobster muscle or an aqueous extract of lobster muscle. It would almost appear that some component of the crustacean muscle may, therefore, have the power to bind metal ions in such a manner as to leave them unavailable to act as lipid oxidation catalysts.

The authors are well aware that in studying rancidity, blended tissue is in many ways different from whole fillets: more water has been added; the tissues and many of the individual cells have been disintegrated and many of the components within the cells have been released and mixed together; the blending also incorporates many small bubbles of atmospheric oxygen. Some caution must be used in trying to apply the findings from these experiments with blended muscle to whole fish or fillets as they are handled under commercial conditions. Nevertheless, it is important to realize the relative activity of the many different heavy metal catalysts including the hematin compounds; to realize the profound effect that many of the nonlipid components of the tissues have on the development of rancidity; and not the least, to learn of the very wide differences in susceptibility of muscles from different species to rancidity induced by the many different metal catalysts.

REFERENCES

ALTMAN, P. L., AND D. S. DITTMER. 1964. Biology data book. Federation Am. Soc. Expt. Biol., Washington, D.C. 633 p.

BANKS, A. 1967. Deteriorative changes in fish oils. *In* M. E. Stansby [ed.] Fish oils. Avi Publ. Co., Westport, Conn. p. 148–163.

BROWN, W. D. 1962. The concentration of myoglobin and hemoglobin in tuna fish. J. Food Sci. 27: 26–28.

BROWN, W. D., LEA. S. HARRIS, AND H. S. OLCOTT. 1963. Catalysis of unsaturated lipid oxidation by iron protoporphyrin derivatives. Arch. Biochem. Biophys. 101: 14–20.

BROWN, W. D., M. MARTINEX, M. JOHNSTONE, AND H. S. OLCOTT. 1962. Comparative biochemistry of myoglobins. J. Biol. Chem. 237: 81–84.

CASTELL, C. H., JILL MACLEAN, AND BARBARA MOORE. 1965. Rancidity in lean fish muscle. IV. Effect of sodium chloride and other salts. J. Fish. Res. Bd. Canada 22: 929–944.

CASTELL, C. H., JILL MACLEAN, BARBARA MOORE, AND WANDA NEAL. 1966. Rancidity in lean fish muscle. V. The effect of amino acids. J. Fish. Res. Bd. Canada 23: 27–43.

CASTELL, C. H., B. A. MOORE, P. M. JANGAARD, AND WANDA E. NEAL. 1966a. Oxidative rancidity in frozen stored cod fillets. J. Fish. Res. Bd. Canada 23: 1385–1401.

CASTELL, C. H., BARBARA MOORE, AND WANDA NEAL. 1966b. Cause and control of spurious thiobarbituric acid values from fish muscle in the presence of inorganic iron salts. J. Fish. Res. Bd. Canada 23: 737–746.

CASTELL, C. H., AND D. M. SPEARS. 1968. Heavy metal ions and the development of rancidity in blended fish muscle. J. Fish. Res. Bd. Canada 25: 639–656.

HOYNAK, P., JR. MS, 1962. The mechanism of the development of rancidity in frozen pork sausage and practicable methods for its inhibition. Ph.D. Thesis. University Microfilms Inc., Ann Arbor, Michigan. 110 p.

MACLEAN, JILL, AND C. H. CASTELL. 1964. Rancidity in lean fish muscle. I. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. J. Fish. Res. Bd. Canada 21: 1345–1359.

MATSUURA, F., AND K. HASHIMOTO. 1959. Chemical studies on the red muscle of fishes. X. A new method for the determination of myoglobin. Bull. Japan. Soc. Sci. Fish. 24: 809–815.

NICOL, J. A. C. 1960. The biology of marine animals. 2nd ed. Sir Isaac Pitman and Sons Ltd., London. 699 p.

OLCOTT, H. S. 1962. Marine products. *In* W. H. Shultz, E. A. Day, and R. O. Sinnhuber [ed.] Lipids and their oxidation. Avi Publ. Co., Westport, Conn. p. 173–189.

STANSBY, M. E., AND W. D. BROWN. 1958. Review of progress on oxidative deterioration of fish and fishery products. Comm. Fish. Rev. 20: 24–27.

TAPPEL, A. L. 1953. Linoleate oxidation catalysts occurring in animal tissues. Food Res. 18: 104–108.

1961. Hematin compounds and lipoxidase as biocatalysts. *In* W. H. Shultz, E. A. Day, and R. O. Sinnhuber [ed.] Lipids and their oxidation. Avi Publ. Co., Westport, Conn. p. 122–138.

1962. Biocatalysts: lipoxidase and hematin compounds. *In* W. O. Lundberg [ed.] Autoxidation and antioxidants, Vol. 1. Interscience Publishers, New York. p. 325–366.

1965. Oxidative reactions and enzymes. *In* R. Kreuzer [ed.] The technology of fish utilization. Fishing News (Books) Ltd., London. p. 29–133.

TARLADGIS, B., B. M. WATTS, M. T. YOUNATHAN, AND L. DUGAN, JR. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chemists Soc. 37: 44–48.

WATTS, B. M. 1954. Oxidative rancidity and discoloration in meats. Advan. Food Res. 5: 1–52.

1961. The role of lipid oxidation in lean tissues in flavour deterioration of meat and fish Proc. Flavour Chem. Symp., Campbell Soup Co., Camden, New Jersey. p. 83–96.

Effective Disinfection of Trout Eggs to Prevent Egg Transmission of *Aeromonas liquefaciens*

T. W. McFADDEN

Laboratory for Experimental Limnology[1]
R.R. #2, Maple, Ont.

McFADDEN, T. W. 1969. Effective disinfection of trout eggs to prevent egg transmission of *Aeromonas liquefaciens*. J. Fish. Res. Bd. Canada 26: 2311–2318.

Treatment of trout eggs with classical disinfectants such as sulfo-merthiolate, merthiolate, and acriflavine proved unreliable for destroying *Aeromonas liquefaciens* on the eggshell. These bacteristatic agents washed free leaving viable bacteria on the shell.

An organic iodine compound, povidone–iodine, was found to be bactericidal at dosages readily tolerated by trout eggs. No reduction in the rate of development was observed after treatment.

Transmission of *A. liquefaciens* from parents to offspring follows the route reported for *Aeromonas salmonicida*. Viable bacterial cells are carried on the outer surface of the shell only, enabling the use of surface disinfection.

Received April 21, 1969

INTRODUCTION

PROTRACTED LETHAL EPIZOOTICS of fry and young fingerling salmonids have been observed in the University of Toronto's stocks at the Laboratory for Experimental Limnology, Maple, Ont. The organism responsible in most instances has been *Aeromonas liquefaciens*. To alleviate this situation, improved hygiene in the holding facilities has been initiated, e.g., incoming water is passed through an ultraviolet sterilizer before reaching the fish tanks. Sterilizing the water, however, does not eliminate infection passed from parents to eggs at the time of spawning. Furunculosis, caused by the closely related *Aeromonas salmonicida*, has been found in the ovaries of artificially infected fish (Mackie et al., 1930). Also the ovaries and testes of naturally infected brook trout have been found to contain the organism (McDermott and Berst, 1968). In the case of *A. salmonicida*, eggs were found to harbour the bacterium externally on the shell (Smith, MS, 1939) but no evidence has been found to indicate the organism exists within the egg (Plehn, 1911; Williamson, 1929; Mackie et al., 1930). Because the pathogen was carried externally only, Gee and Sarles (1942) found they could disinfect furunculosis-infected trout eggs with a number of chemical substances. These were sulfo-merthiolate, mercuric chloride, mercarbolide, acriflavine, gentian violet, sodium and calcium hypochlorite, chlorozene, azochloramid, and perhaps formaldehyde.

[1]Jointly operated by the Ontario Department of Lands and Forests, and the Department of Zoology, University of Toronto, Toronto, Ont.

Printed in Canada (J1450)

The primary purpose of the present study was to determine whether *A. liquefaciens* could be transmitted from parent to offspring through the egg. The second objective was to determine whether a practical disinfection technique could be established to break such a link.

MATERIALS AND METHODS

Disinfectants Tested

Four disinfectants were tested in an attempt to find a sure and safe method of treating trout eggs. Sulfo-merthiolate[2] was the first disinfectant studied. However, among other faults this compound is no longer manufactured. Merthiolate,[3] a closely related mercurial in current production was tried next. Neutral acriflavine formerly produced by Abbot Laboratories but now available only from European pharmaceutical houses or importers such as H. Reisman Corp.[4] was highly recommended (Smith, MS, 1939) and was tried after the mercurials. Finally Povidone–Iodine (polyvinylpyrrolidone–iodine, PVP-I was discovered and tried. This compound is available commercially under the trade names Bridine[5] and Betadine.[6]

Bacterial Culture

Two cultures were utilized in this study, *A. liquefaciens* (MS 68) recovered from rainbow trout at the Maple Laboratory, and *A. hydrophila* (OAC 352) received through the courtesy of Professor L. A. McDermott, University of Guelph, Guelph, Ont. Only the former organism was utilized in the assessment of the disinfecting properties of sulfo-merthiolate, merthiolate, and neutral acriflavine.

The pure cultures were maintained on trypticase soy agar (BBL). Transfers were made to trypticase soy agar subculture or trypticase soy broth (BBL) 24–48 hr before use as infecting agents.

Identification of recovered organisms was based on the presumptive scheme of Bullock (1961). Before classification as *A. liquefaciens* all cultures, including *A. hydrophila*, were found to possess all the characteristics ascribed to *A. liquefaciens* in this scheme. In addition, cultures were determined to be indole (Kovăc's test in tryptophane broth), and Voges-Proskauer (Coblentz test in MR-VP broth) positive after 48 hr. Distinction between the two strains was difficult but was made on the basis of results in the glucose broth of Bullock (1961). After 16 hr the OAC 352 culture produced very little gas and considerable acid, whereas the MS 68 culture produced considerable gas and little acid.

Effect of Disinfectants on Bacterial Viability

Assays were performed to determine the minimum concentration of the disinfectant required to effect a total mortality on *A. liquefaciens* culture in 10 min at 10 C. For this test 15 ml of a 24-hr broth culture were centrifuged 2 min at 2000 rpm, the supernatant was drawn off, then the cells were resuspended in the same volume of sterile tap water before disinfection. After disinfection the culture was centrifuged and washed three times to remove the disinfectant. A loopful of packed cells was then introduced to determine if viable cells remained. The lowest concentration of disinfectant that yielded sterile culture after 72 hr represented the minimum bactericidal dosage.

Surface Disinfection of Eggs

Eggs were dipped in freshly prepared solutions of disinfectant for 10 min at 10 C after which the disinfectant was removed with three successive rinses in sterile tap water (as many as

---

[2]Eli Lily and Company (Canada) Ltd., Toronto, Ont.
[3]Eli Lily and Company (Canada) Ltd., Toronto, Ont.
[4]H. Reisman Corp., 114 Liberty St., New York, N.Y. 10006.
[5]British Drug Houses (Canada) Ltd. Toronto, Ont.
[6]Purdue Frederick Company (Canada) Ltd., Toronto, Ont.

15 washes were used to remove bound acriflavine.) Modification of the Booth and Schrieber[7] (1925) electromicroqualitative method for the determination of mercury[8] revealed the absence of mercury after three washes. Absence of colour was utilized as the criterion for removal of disinfectant with PVP-I and acriflavine. Samples of 10 eggs per trial were subsequently aseptically transferred to broth for sterility tests. Undisinfected egg samples and uninoculated broth samples were retained as positive and negative controls.

Disinfectant Egg Toxicity

After determining the minimum bactericidal dosage for bacteria using PVP-I the toxicity to eyed eggs was determined. Duplicate lots of 25 eyed rainbow trout eggs were subjected to increasing concentrations of aqueous PVP-I. After 10 min the eggs were thoroughly rinsed in sterile water to remove the PVP-I. Incubation at 9–11 C followed. A daily mortality record was maintained until the last egg hatched.

Bacterial Transmission

Rainbow trout males were prepared for stripping, after anaesthetization, by thoroughly wiping the anal and peduncular area with full strength PVP-I (10% PVP-I or 1% I_2) and allowing it to dry. Milt was then collected in sterile test tubes. A portion was immediately inoculated into broth for culture and the remainder was split into two samples. One half was vigourosly mixed with scrapings from a 48-hr growth of cultute on agar, the other half was unmodified. Both tubes were set aside for 30 min to allow the bacteria an apportunity to invade the spermatozoa. Females were anaesthetized, disinfected like the males, and stripped of eggs. The eggs were divided into three lots in sterile beakers. Lot 1 (green) was unmodified, lot 2 (infected) was fertilized with infected milt, and lot 3 (normal) was fertilized with normal milt. After removal of excess milt with sterile wash water the eggs were sampled. Ten eggs were removed from each lot and inoculated into broth. These eggs would indicate the external flora of the shell. A second series of samples was taken from each lot in which the shells were aseptically perforated prior to inoculation in broth. This series would allow development of the flora of the interior. After thorough shaking and incubation for 1 hr all unperforated eggs were removed.

Disinfection of the green, infected, and uninfected eggs followed. These were treated to 1% PVP-I for 10 min at 10 C. Specimens were then washed in three changes of sterile tap water and sampled exactly as the three groups above. The unperforated series when cultured would indicate whether the shell surface had been properly sterilized. If the disinfectant properly sterilized the egg surface then the results of culture from the perforated series would indicate whether or not the organism penetrated the egg.

RESULTS AND DISCUSSION

Merthiolate and Sulfo-Merthiolate Disinfection

Early in this investigation the results of Gee and Sarles (1942) on *A. salmonicida* disinfection on trout eggs were taken for granted. However, consistent contradictions with previous literature on the manner of infection of the eggs required that the bacteriological potency of sulfo-merthiolate be redetermined. Plehn (1911), Williamson (1929), and Mackie et al. (1930) all

[7]Cited in Hillebrand et al. (1953, p. 220, subscript 36).

[8]Ten egg lots were ground in a tissue grinder, 5 ml of 20% nitric acid was added and the acid mash allowed to incubate 20 min. After incubation a drop of mash was placed on a microscope slide and electrolysed 5 min with a very fine copper wire cathode and a platinum anode. Voltage was supplied by a single dry cell. After electrolysis the cathode was washed under a tap and examined under low power on a microscope. If mercury is present an amalgam can be seen on the tip. Sensivitity is reported to be 1 part in 2 million.

concurred that there was no evidence that *A. salmonicida* was carried within the trout egg. However, the present author repeatedly isolated *A. liquefaciens* from broth cultures of perforated eggs, previously disinfected with sulfo-merthiolate. This meant that if sulfo-merthiolate properly disinfected the egg surface this organism could be transmitted internally.

Trials to assess the disinfecting ability of sulfo-merthiolate on *A. liquefaciens* culture revealed a complete inability to destroy the bacteria. An increase in disinfectant concentration of three times (.05%) and time of disinfection of $2\frac{1}{2}$ times (25 min) that recommended by Gee and Sarles for *A. salmonicida* still left viable bacteria (Table 1). Similar trials with merthiolate produced the same results. Substitution of a Maple strain of *A. salmonicida* in the disinfection indicated this species is as unaffected by sulfo-merthiolate as *A. liquefaciens*. Finally a trial was performed according to Gee and Sarles (1942), that is, extracting a sample of culture and disinfectant, after disinfection, and inoculating into broth. Transferring a loopful of *A. liquefaciens* and 0.02% sulfo-merthiolate solution from the disinfection bath to sterile broth did not appear to contaminate the medium with *A. liquefaciens*.

TABLE 1. Disinfectant ability of sulfo-merthiolate, merthiolate and neutral acriflavine on *A. liquefaciens*.

Aqueous culture

Disinfection time (*min*)	Sulfo-merthiolate (%)		Merthiolate (%)		Acriflavine (%)	
	0.02	0.04	0.02	0.04	0.02	0.04
0	+	+	+	+	+	+
5	+	+	+	+	+	
10	+	+	+	+	+	–[a]
15	+	+	+	+		
20	+	+	+	+		
25	+	+	+	+	–[a]	–[a]

Infected raindow trout eggs

Disinfection time (*min*)	Sample	24-hr culture (A.I.)	Density (McFarland tubes)	
			24 hr	48 hr
	Fertilized washed unperforated	+		#5–#6
	Fertilized washed perforated	+		#4
10	0.04% merthiolate unperforated	+		#5–#6
10	0.04% merthiolate perforated	+		#5–#6
10	0.02% acriflavine unperforated	+	<#1	#1
10	0.02% acriflavine perforated	+	<#1	#1
25	0.02% acriflavine unperforated	–[a]	<#1	#1
25	0.02% acriflavine perforated	–[a]	<#1	#1–#2

[a]Negative after 24 hr – residual acriflavine not totally removed after nine rinsings. Positive 72 hr after removal of residual.

It would appear, therefore, that what Gee and Sarles regarded as bactericidal in its effect was only bacteriostatic. Wilson and Miles (1964) contains an excellent review of disinfectants. These authors point out that the mercurials are bacteriostatic only. The Gee and Sarles' method could not distinguish between dead and bacteriostatically inhibited cells. They realized the possibility of bacteriostasis and attempted to counter it by making serial subcultures. In the present study disinfectant was removed from bacterial cells and eggs, eliminating any inhibiting effect and measuring bactericidal activity only. The danger of the older technique is that after disinfection eggs would be returned to incubators that suspend them in water slowly washing their surface. In this instance bacteriostatic agents, such as merthiolate, would be removed before the pathogens died.

Acriflavine Disinfection

After the merthiolates, neutral acriflavine was tested as a disinfectant for *A. liquefaciens* cultures. Like the mercurials it was widely recommended for egg disinfection (Fish and Wildlife Service, 1955; Leitritz, 1959; Smith, MS, 1939; Gee and Sarles, 1942). Cultures were disinfected with 0.02% (recommended by Leitritz, 1959) and 0.04% for 10 and 25 min. Initially acriflavine appeared more useful than the mercurials. Bacterial growth stopped after 0.02% treatment for 25 min (Table 1). However, the disinfectant did not wash out readily. If it was removed by 15 sterile rinses over a 2-hr period growth of *A. liquefaciens* occurred within 72 hr. Acriflavine, therefore, is also bacteriostatic and susceptible to washing out.

Povidone–Iodine Disinfection

A search for a safe positive disinfectant (a bactericidal one) revealed PVP-1. This proved far superior to the three previously discussed disinfectants (Table 2).

Table 2. Bactericidal dosage of povidone–iodine on *A. liquefaciens* disinfected 10 min at 10 C.

Dosage (%)	Aqueous culture 72 hr subculture	Contaminated eggs 72 hr subculture
0.05	+	
0.10	+	
0.25	+	
0.30	+	+
0.50	–	–
0.80		–
1.00		–
1.20		–
Control (infected)	+	+
Negative control	–	–

After determining the dosage required to kill *A. liquefaciens* alone in water it was necessary to find whether or not any increased dosage was required in the presence of proteinaceous eggshells. Literature available from one of the commercial supplies (Anon.) indicated little reduction in activity in the presence of blood or serum. From Table 2 it is noted that 0.5% PVP-I is required to kill *A. liquefaciens* alone or in the presence of trout eggs.

Gee and Sarles (1942) reported the lethal dose of free iodine for brook and brown trout was 0.05%. They also reported protracted incubation was required after disinfecting with halogens. Table 3 illustrates that PVP-I

TABLE 3. Povidone–iodine toxicity on eyed rainbow trout eggs.

	Disinfectant concentration (%)							
	Control	0.5	1.0	2.0	2.5	3.0	4.0	5.0
Mean survival time (*hr*)	–	–	–	–	–	<24	<24	<24
Median hatch time (*days*)	43	43	43	43	43	–	–	–
Sample size	100	50	50	50	50	50	50	50
% mortality at hatch	0	2	2	2	0	100	100	100

is not toxic up to doses of 2.5–3.0% (.25–.30% free I_2) for eyed rainbow trout eggs. Furthermore no extension was observed in the incubation period required for PVP-I treated eggs.

TRANSMISSION OF *A. LIQUEFACIENS*

Only two mature rainbow trout with a natural *A. liquefaciens* infection in the ovaries have been examined when viable eggs could be collected and properly disinfected (Table 4). In both instances the eggs retained viable bacterial cells in spite of three vigorous washings with sterile tap water. Disinfection of the outer surface with 1% PVP-I followed by thorough washing to remove the disinfectant,, however, killed all viable cells. If these eggs were disinfected, washed, perforated, and then inoculated into broth the broth remained sterile.

Similarly fertilization of green eggs with infected milt of *A. liquefaciens* produced the same effect, that is, infection of the outer surface of the egg but no penetration into the egg itself (Table 4). Therefore, *A. liquefaciens* appears to be carried mechanically on the outer surface of the egg shell as reported previously for *A. salmonicida*.

CONCLUSIONS

The commonly used bacterial egg disinfectants, sulfo-merthiolate, merthiolate, and neutral acriflavine were found to be generally unsatisfactory for disinfecting trout eggs. The first two are

TABLE 4. Transmission of infection from parent to eggs. Disinfection with 1% povidone–iodine.

Naturally infected female			
Sample	Sample size	24 hr culture	Trials
Ovarian fluid	2 fish	+ *A. liquefaciens*	2
Green eggs external	10 eggs	+	2
Green eggs internal	10	+	2
Milt	1 ml	–	2
Fertilized eggs exterior	10	+	2
Fertilized eggs interior and exterior	10	+	2
Fertilized eggs disinfected exterior	10	–	2
Fertilized eggs disinfected exterior and interior	10	–	2

Artificially infected milt			
Sample	Sample size	*A. liquefaciens*[a]	*A. hydrophila*[b]
Green eggs exterior	10 eggs	–	–
Green eggs exterior and interior	10	–	–
Milt uninfected	1 ml	–	–
Milt infected	1 ml	+	+
Fertilized uninfected exterior	10 eggs	–	–
Fertilized uninfected exterior and interior	10	–	–
Fertilized infected exterior and interior	10	+	+
Fertilized uninfected disinfected exterior	10	–	–
Fertilized uninfected disinfected exterior and interior	10	–	–
Fertilized infected disinfected exterior	10	–	–
Fertilized infected disinfected exterior and interior	10	–	–

[a]Triplicate trials performed.
[b]One trial only performed.

bacteriostatic and readily removed by running water. Acriflavine, although not as easily removed, is also bacteriostatic and could be washed away before the contaminating bacteria were killed.

Povidone-iodine (PVP-I) was found to be an effective egg disinfectant when eggs were immersed in a 1% solution for 10 min at 10 C. It was bactericidal, provided an adequate safety margin to rainbow trout, did not inhibit hatching, and was readily removed.

Disinfection of the outer surface of eyed trout eggs has been shown to prevent transmission of *A. liquefaciens* from either parent to the offspring at the time of fertilization.

It has been suggested to the author that PVP-I might be useful to study the intra-egg transmission of certain bacterial diseases like kidney disease. This possibility appears very likely but no bactericidal claims are made in this study for PVP-I on organisms other than the aeromonads tested.

ACKNOWLEDGMENTS

The author expresses sincere appreciation to Dr F. E. J. Fry, Director of the Laboratory for Experimental Limnology, for his continued interest and encouragement in this Laboratory's fish disease abatement programs. Professor L. A. McDermott, Department of Microbiology, University of Guelph, kindly supplied the *Aeromonas hydrophila* culture, OAC 352. Thanks are also extended to Mr A. H. Berst, Ontario Department of Lands and Forests, Research Branch, and to Dr H. H. Harvey, Department of Zoology, University of Toronto, for reviews of the manuscript. This investigation was supported in part by a grant from the National Research Council of Canada and the Fisheries Research Board of Canada.

REFERENCES

ANON. Bridine (Providone–Iodine) a unique non-selective microbicide. Product Information Circular British Drug Houses (Canada) Toronto. 4 p.

BULLOCK, G. L. 1961. A schematic outline for the presumptive identification of bacterial diseases of fish. Progressive Fish-Culturist 23: 147–151.

GEE, L. L., AND W. B. SARLES. 1942. The disinfection of trout eggs contaminated with *Bacterium salmonicida*. J. Bacteriol. 44: 111–126.

HILLEBRAND, W. F., G. E. F. LUNDELL, H. A. BRIGHT, AND J. I. HOFFMAN. 1953. Applied inorganic analysis. 2nd ed. John Wiley and Sons Inc., New York. 1034 p.

LEITRITZ, E. 1959. Trout and salmon culture — hatchery methods. Fish. Bull. 107, Dept. Fish Game, Calif. 169 p.

MACKIE, J. J., J. A. ARKWRIGHT, T. E. PRYCE-TAMMATT, J. C. MOTTRAN, W. D. JOHNSON, W. J. M. MENZIES, AND W. MARTIN. 1930. Interim report of Furunculosis Committee. H.M. Stationery Office, Edinburg. 65 p.

MCDERMOTT, L. A., AND A. H. BERST. 1968. Experimental plantings of brook trout (*Salvelinus fontinalis*) from furunculosis-infected stock. J. Fish. Res. Bd. Canada 25: 2643–2649.

PLEHN, M. 1911. Die furunculose der salmoniden. Zentr. Bakteriol. Parasitenk. Abt. I Orig. 60: 607–624. Cited in Gee and Sarles (1942).

SMITH, W. W. MS, 1939. The disinfecting action of neutral acriflavine on *Bacterium salmonicida*. Ph.D. Thesis. Univ. Wisconsin, Madison, Wis. 61 p.

WILLIAMSON, I. J. F. 1929. Further observations on furunculosis of salmonidae. Fish. Res. Bd. Scotland Salmon Fish. 1. H.M. Stationery Office, Edinburg. 12 p.

WILSON, G. S., AND A. A. MILES. 1964. Topley and Wilson's principles of bacteriology and immunity. Vol. 1. 5th ed. Edward Arnold Ltd., London. 1191 p.

Preliminary Report on the Parasites of Certain Marine Fishes of British Columbia

HISAO P. ARAI[1]

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

ARAI, HISAO P. 1969. Preliminary report on the parasites of certain marine fishes of British Columbia. J. Fish. Res. Bd. Canada 26: 2319–2337.

The results of a study to assess the kinds and degrees of parasitism that exist in or on inshore fishes of British Columbia are presented. The report contains a listing of 68 taxa of parasites recovered from a total of 814 host specimens representing 61 species.

Observations on host–parasite relationships made during the course of the study are discussed.

Received February 21, 1969

INTRODUCTION

FOR PERIODS DURING THE SUMMERS OF 1964, 1965, and 1966, a survey of the parasites of fishes of Burke Channel, British Columbia, was conducted in conjunction with other studies on the early sea life of pink salmon, *Oncorhynchus gorbuscha* (Walbaum). This study was made to assess the kinds and degrees of parasitism that exist in the resident fishes of the area and to determine whether these fishes act as reservoir hosts for parasites occurring in or on the young pink salmon migrating through Burke Channel–Fitz Hugh Sound on their way to the open sea. A separate study of the parasites of pink salmon was carried out in 1964 by Boyce (1969).

The results of two other collections, made during 1963 on the southern coast of British Columbia (Bowen Island and Departure Bay), are included in this report. Although my report (Arai, 1967) shows that definite differences can occur in the geographical distribution of fish parasites, the three collections have been grouped as a single unit to minimize possible duplication in multiple publications.

A total of 814 fishes representing 61 species from 47 genera in 21 families were examined in the course of the work.

MATERIALS AND METHODS

Host specimens taken in Burke Channel were usually collected with a purse seine along with samples of fingerling pink salmon. However, additional materials were taken with an otter trawl, hook and line, poisons, and a minnow seine. The collection at Bowen Island was made with the use of rotenone to depths of some 60 ft; the one at Departure Bay was made in a *Zostera marina* bed with a beach seine. The fishes collected at all localities were maintained in live tanks

[1]Mailing address: Department of Biology, University of Calgary, Calgary, Alta.

Printed in Canada (J1386)

or were frozen until examined for parasites. Living host specimens were usually examined within 48 hr from the time of capture; those that were frozen were examined up to 2 months later. Except for the species of *Sebastodes* that were identified by John Thomson of the Biological Station, Nanaimo, host fishes were identified by use of keys in Clemens and Wilby (1961); however, the names used in the present report follow those suggested by Bailey et al. (1960).

The parts of the fishes examined for parasites (metazoan only) included the entire digestive tract, gills, body cavity, and the external surface of the body. The sex, standard length, and general condition of the fishes were also noted at the time of examination.

Examinations of fishes were made with the use of a stereoscopic microscope at magnifications of 6–60×. The parasites recovered were heat- or cold-killed and preserved in a mixture of alcohol–formalin–acetic acid or in 70% ethanol. Representative specimens of trematodes, cestodes, and acanthocephalans from the various collections were stained with haematoxylin or with acetocarmine and mounted in Canada balsam. Nematodes were cleared in lactophenol or in glycerol and identified in temporary mounts in these clearing agents. Identifications of the Copepoda were made by Dr Z. Kabata of the Fisheries Research Board of Canada Biological Station, Nanaimo, and by Dr L. S. Roberts, University of Massachusetts, Amherst, Mass. The gyrodactyloid Monogenea have been submitted to Mr C. E. Price, Millersville State College, Millersville, Pennsylvania, for further identification. All specimens of *Philonema* were referred to Dr A. K. M. Bashirullah, formerly with the Department of Zoology, University of British Columbia. Yamaguti's (1958, 1959, 1961, 1963a, 1963b) volumes of *Systema Helminthum* were used extensively in identifying the collections to the familial and generic levels.

RESULTS

The results appear in two lists, arranged according to the following plan:

List 1 — Parasites are listed under host species, which are arranged in phylogenetic order according to Bailey et al. (1960). The parasites are grouped into taxa for each host species from which they were recovered. Numbers after *host* species indicate number of fish examined and numbers infected, e.g., Pacific herring (29; 25). Numbers after *parasite* species indicate number of fish in which the parasite was found (incidence of infection) and range in intensity of infection, e.g., *Lecithaster gibbosus* (20; 1–20). A single number indicates incidence of infection only, e.g., Gyrodactyloidea (9; –).

List 2 (p. 2328) — Hosts are grouped by parasite species. Numbers as fractions indicate incidence of infection in host species. The second number indicates the range in intensity of infection, followed by locations from which parasites were recovered,, e.g., (p. 2328) *Hemilepidotus hemilepidotus* (8/22; 1–12); intestine.

LIST 1. PARASITES BY HOST SPECIES

CLUPEIFORMES

CLUPEIDAE

Clupea harengus pallasi Valenciennes, Pacific herring (29; 25)

Digenea:	*Lecithaster gibbosus* (Rud., 1802) (20; 1–22)
	Parahemiuris merus (Linton, 1910) (3; 2–5)
	Pentagramma petrowi (Layman, 1930) (3; 1–3)
Monogenea:	Gyrodactyloidea (9; –)
Cestoda:	*Phyllobothrium* sp. (larval) (10; 1–2)
Nematoda:	*Anisakis* sp. (larval) (4; 2–17)
	Contracaecum sp. (larval) (3; 1–10)

Copepoda: *Caligus clemensi* Parker and Margolis, 1964 (2; 1)
Parabomolochus cuneatus (Fraser, 1920) (1; 2)

SALMONIDAE

Oncorhynchus gorbuscha (Walbaum), pink salmon (81; 28)
Digenea: *Lecithaster gibbosus* (Rud., 1802) (18; 1–9)
Cestoda: *Phyllobothrium* sp. (larval) (10; 1–2)
Nematoda: *Anisakis* sp. (larval) (2; 1)
Contracaecum sp. (larval) (2; 1)

Oncorhyochus keta (Walbaum), chum salmon (34; 18)
Digenea: *Galactosomum* sp. (larval) (1; 2)
Lecithaster gibbosus (Rud., 1802) (16; 1–15)
Cestoda: *Phyllobothrium* sp. (larval) (2; 1)
Nematoda: *Anisakis* sp. (larval) (2; 1–2)
Contracaecum sp. (larval) (3; 1–7)
Acanthocephala: *Neoechinorhynchus rutili* (Mueller, 1780) (1; 1)

Oncorhynchus kisutch (Walbaum), coho salmon (60; 40)
Digenea: *Crepidostomum farionis* (Mueller, 1784) (14; 1–23)
Lecithaster gibbosus (Rud., 1802) (27; 1–40)
Parahemiuris merus (Linton, 1910) (5; 1–8)
Monogenea: Gyrodactyloidea (1; 1)
Cestoda: *Diphyllobothrium* sp. (larval) (9; 1–2)
Phyllobothrium sp. (larval) (10; 1–2)
Proteocephalus sp. (larval) (2; 1)
Nematoda: *Anisakis* sp. (larval) (5; 1–5)
Ascarophis sebastodis Olsen, 1952 (7; 1–7)
Capillaria sp. (2; 1–3)
Contracaecum sp. (larval) (2; 1–2)
Cucullanus sp. (1; 1)
Philonema sp. (larval) (10; –)
Acanthocephala: *Corynosoma* sp. (juvenile) (1; 1)
Echinorhynchus gadi Mueller, 1776 (1; 1)
Neoechinorhynchus rutili (Mueller, 1780) (4; 1–6)
Copepoda: *Salmincola californiensis* Dana, 1852 (1; 1)

Oncorhynchus nerka (Walbaum), sockeye salmon (8; 8)
Digenea: *Genolinea laticauda* Manter, 1925 (1; 1)
Lecithaster gibbosus (Rud., 1802) (7; 4–43)
Parahemiuris merus (Linton, 1910) (1; 4)
Cestoda: *Diphyllobothrium* sp. (larval) (7; 1–3)
Phyllobothrium sp. (larval) (3; 2–4)
Nematoda: *Anisakis* sp. (larval) (2; 1)
Ascarophis sebastodis Olsen, 1952 (1; 1)
Contracaecum sp. (larval) (2; 1)
Acanthocephala: *Neoechinorhynchus rutili* (Mueller, 1780) (3; 1–5)

Oncorhynchus tshawytscha (Walbaum), chinook salmon (1; 1)
Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 1)
Cestoda: *Phyllobothrium* sp. (larval) (1; 1)
Nematoda: *Capillaria* sp. (1; 2)
Contracaecum sp. (larval) (1; 3)
Philonema sp. (larval) (1; 2)
Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (1; 1)

Salvelinus malma (Walbaum), Dolly Varden (2; 2)
Digenea: *Crepidostomum farionis* (Mueller, 1784) (1; 1)
Lecithaster gibbosus (Rud., 1802) (2; 10–21)
Tubulovesicula lindbergi (Layman, 1930) (2; 1–2)

Cestoda: *Phyllobothrium* sp. (larval) (1; 1)
Nematoda: *Contracaecum* sp. (larval) (1; 1)
Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (1; 6)

OSMERIDAE

Thaleichthys pacificus (Richardson), eulachon (7; 3)
Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 2)
Cestoda: *Phyllobothrium* sp. (larval) (1; 1)
Nematoda: *Contracaecum* sp. (larval) (1; 1)

GADIFORMES

GADIDAE

Gadus macrocephalus Tilesius, Pacific cod (18; 18)
Digenea: *Lecithaster gibbosus* (Rud., 1802) (18; 3–51)
Parahemiuris merus (Linton, 1910) (8; 1–6)
Cestoda: *Phyllobothrium* sp. (larval) (5; 1–2)
Nematoda: *Anisakis* sp. (larval) (1; 1)
Theragra chalcogramma (Pallas), walleye pollack (2; 2)
Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 6)
Lepidapedon microcotyleum Dollfus, 1953 (1; 1)
Neopodocotyloides sinusaccus (Ching, 1960) (1; 15)
Cestoda: *Bothriocephalus scorpii* (Mueller, 1776)(larval) (2; 2–3)
Nybelinia surmenicola Okada, 1929 (larval) (1; 26)
Phyllobothrium sp. (larval) (1; 1)
Nematoda: *Anisakis* sp. (larval) (1; 1)
Contracaecum sp. (larval) (1; 13)
Porrocaecum sp. (larval) (1; 14)
Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (2; 7–41)

GASTEROSTEIFORMES

GASTEROSTEIDAE

Gasterosteus aculeatus Linnaeus, threespine stickleback (23; 23)
Digenea: *Derogenes varicus* (Mueller, 1784) (1; 3)
Lecithaster gibbosus (Rud., 1802) (5; 1–4)
Neopodocotyloides sinusaccus (Ching, 1960) (1; 1)
Monogenea: Gyrodactyloidea (21; –)
Cestoda: *Bothriocephalus scorpii* (Mueller, 1776) (larval) (3; 2–4)
Phyllobothrium sp. (larval) (8; 1–7)
Nematoda: *Anisakis* sp. (larval) (4; 1)
Copepoda: *Ergasilus auritus* Markevich, 1940 (22; 4–20)

AULORHYNCHIDAE

Aulorhynchus flavidus Gill, tube-snout (5; 5)
Digenea: *Lecithaster gibbosus* (Rud., 1802) (4; 2–50)
Podocotyle atomon (Rud., 1802) (1; 8)
Cestoda: *Bothriocephalus* sp. (larval) (1; 2)
Phyllobothrium sp. (larval) (1; 1)
Nematoda: *Anisakis* sp. (larval) (2; 1–2)
Ascarophis sebastodis Olsen, 1952 (1; 1)
Contracaecum aduncum (Rud., 1802) (larval) (2; 8–12)

SYNGNATHIDAE

Syngnathus griseolineatus Ayres, bay pipefish (36; 25)

Digenea:	*Lecithaster gibbosus* (Rud., 1802) (8; 1–2)
	Neopodocotyloides sinusaccus (Ching, 1960) (1; 2)
	Opechona occidentalis Montgomery, 1957 (1; 1)
	Parahemiuris merus (Linton, 1910) (1; 1)
	Podocotyle endophrysi Park, 1937 (4; 1–2)
	Tubulovesicula lindbergi (Layman, 1930) (2; 1)
Monogenea:	Gyrodactyloidea (1; –)
Cestoda:	*Phyllobothrium* sp. (larval) (1; 1)
Nematoda:	*Anisakis* sp. (larval) (4; 1–3)
	Contracaecum sp. (larval) (2; 1)
Copepoda:	*Haemobaphes* sp. (juvenile) (8; 1)

PERCIFORMES

EMBIOTOCIDAE

Brachyistius frenatus Gill, kelp perch (4; 1)

Digenea:	*Lecithaster gibbosus* (Rud., 1802) (1; 2)

Cymatogaster aggregata Gibbons, shiner perch (138; 123)

Digenea:	*Galactosomum* sp. (larval) (2; 1–8)
	Lecithaster gibbosus (Rud., 1802) (22; 1–2)
	Neozoogonus californicus Arai, 1954 (21; 1–80)
	Telolecithus pugetensis Lloyd and Guberlet, 1932 (20; 1–100)
	Tubulovesicula lindbergi (Layman, 1930) (1; 2)
Monogenea:	Gyrodactyloidea (29; –)
Cestoda:	*Phyllobothrium* sp. (larval) (5; 1–3)
Nematoda:	*Contracaecum* sp. (larval) (1; 2)
	Cucullanus sp. (42; 1–11)
Acanthocephala:	*Corynosoma* sp. (juvenile) (1; 1)
	Echinorhynchus gadi Mueller, 1776 (1; 1)
Copepoda:	*Ergasilus turgidus* Fraser, 1920 (61; 1–105)
	Haemobaphes diceraus Wilson, 1917 (13; 1–3)
	Parabomolochus cuneatus (Fraser, 1920) (30; 1–4)
Isopoda:	*Livoneca californica* Schioedte and Meinert, 1884 (11; 1)

Rhacochilus vacca (Girard), pile perch (2; 0)

GOBIIDAE

Coryphopterus nicholsi (Bean), bluespot goby (43; 31)

Digenea:	*Lecithaster gibbosus* (Rud., 1802) (1; 3)
Cestoda:	*Phyllobothrium* sp. (larval) (1; 3)
Nematoda:	*Contracaecum* sp. (larval) (30; 1–3)

SCORPAENIDAE

Sebastodes alutus (Gilbert), Pacific ocean perch (3; 3)

Digenea:	*Podocotyle* sp. (immature) (2; 3–5)
Cestoda:	*Bothriocephalus* sp. (larval) (1; 1)
Nematoda:	*Anisakis* sp. (larval) (1; 1)

Sebastodes caurinus (Richardson), copper rockfish (13; 13)

Digenea:	Digenea (immature) (2; 1)
	Opechona occidentalis Montgomery, 1957 (6; 1–18)
	Stephanostomum casum (Linton, 1910) (3; 1–9)
Monogenea:	*Megalocotyle marginata* Folda, 1928 (2; 2–4)
	Microcotyle sebastis Goto, 1894 (5; 1–6)

Nematoda: *Ascarophis sebastodis* Olsen, 1952 (11; 1–120)
Contracaecum sp. (larval) (6; 1–2)
Cucullanus smedleyi Campana-Rouget, 1957 (4; 1)

Sebastodes diploprora (Gilbert), splitnose rockfish (1; 1)
Cestoda: *Phyllobothrium* sp. (larval) (1; 3)

Sebastodes elongatus (Ayres), greenstriped rockfish (3; 3)
Digenea: Digenea (immature) (1; 2)
Opechona occidentalis Montgomery, 1957 (1; 1)
Monogenea: *Microcotyle sebastis* Goto, 1894 (2; 6–12)
Nematoda: *Ascarophis sebastodis* Olsen, 1952 (1; 2)

Sebastodes maliger (Jordan and Gilbert), quillback rockfish (7; 5)
Digenea: *Deretrema cholaeum* McFarlane, 1936 (3; 1–2)
Helicometra sp. (1; 2)
Neopodocotyloides sinusaccus (Ching, 1960) (1; 2)
Cestoda: *Phyllobothrium* sp. (larval) (1; 1)

Sebastodes melanops (Girard), black rockfish (1; 0)

ANOPLOPOMATIDAE

Anoplopoma fimbria (Pallas), sablefish (1; 0)

HEXAGRAMMIDAE

Hexagrammos decagrammus (Pallas), kelp greenling (1; 1)
Copepoda: *Haemobaphes* sp. (juvenile) (1; 14)

Hexagrammos stelleri Tilesius, whitespotted greenling (3; 3)
Digenea: *Anisorchis opisthorchis* Polyansky, 1955 (1; 11)
Lecithaster gibbosus (Rud., 1802) (3; 1–5)
Lecithophyllum anteroporum Margolis, 1958 (1; 1)
Neopodocotyloides sinusaccus (Ching, 1960) (1; 4)
Parahemiuris merus (Linton, 1910) (1; 3)
Podocotyle sp. (immature) (1; 3)
Cestoda: *Phyllobothrium* sp. (larval) (1; 3)

Hexagrammos superciliosus (Pallas), rock greenling (31; 31)
Digenea: *Anisorchis opisthorchis* Polyansky, 1955 (7; 1–7)
Galactosomum sp. (larval) (2; 1)
Genolinea laticauda Manter, 1925 (4; 1–2)
Lecithaster gibbosus (Rud., 1802) (16; 1–17)
Lecithophyllum anteroporum Margolis, 1958 (1; 1)
Neopodocotyloides sinusaccus (Ching, 1960) (1; 1)
Monogenea: *Microcotyle sebastis* Goto, 1894 (3; 2–9)
Cestoda: *Bothriocephalus scorpii* (Mueller, 1776) (1; 1)
Phyllobothrium sp. (larval) (21; 2–32)
Nematoda: *Anisakis* sp. (larval) (7; 1–2)
Ascarophis sebastodis Olsen, 1952 (1; 4)
Contracaecum sp. (larval) (16; 1–7)

Ophiodon elongatus Girard, lingcod (34; 32)
Digenea: *Lecithaster gibbosus* (Rud., 1802) (27; 1–52)
Parahemiuris merus (Linton, 1910) (2; 1)
Rhipidocotyle elongata McFarlane, 1936 (1; 6)
Stephanostomum tristephanum McFarlane, 1936 (1; 8)
Tubulovesicula lindbergi (Layman, 1930) (2; 1–52)
Cestoda: *Nybelinia surmenicola* Okada, 1929 (larval) (1; 115)
Phyllobothrium sp. (larval) (14; 1–2)
Nematoda: *Anisakis* sp. (larval) (1; 315)
Porrocaecum sp. (larval) (3; 1–3)

COTTIDAE

Artedius fenestralis Jordan and Gilbert, padded sculpin (2; 2)
- Digenea: *Genolinea laticauda* Manter, 1925 (2; 1)

Artedius harringtoni Starks, scalyhead sculpin (11; 9)
- Digenea: *Stephanostomum casum* (Linton, 1910) (1; 1)
- Cestoda: *Bothriocephalus scorpii* (Mueller, 1776) (larval) (6; 1–2)
- Nematoda: *Ascarophis sebastodis* Olsen, 1952 (1; 22)
- *Contracaecum* sp. (larval) (3; 1)

Blepsias cirrhosus (Pallas), silverspotted sculpin (6; 6)
- Digenea: *Genolinea laticauda* Manter, 1925 (4; 1–3)
- *Lecithaster gibbosus* (Rud., 1802) (1; 3)
- *Neopodocotyloides sinusaccus* (Ching, 1960) (4; 1–4)
- *Podocotyle* sp. (immature) (1; 1)
- Cestoda: *Phyllobothrium* sp. (larval) (2; 1)
- Nematoda: *Anisakis* sp. (larval) (2; 1)
- Copepoda: *Haemobaphes* sp. (juvenile) (2; 2)

Chitonotus pugetensis (Steindachner), roughback sculpin (2; 2)
- Nematoda: *Capillaria* sp. (1; 3)
- *Contracaecum* sp. (larval) (2; 3)

Dasycottus setiger Bean, spinyhead sculpin (2; 1)
- Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 1)
- *Lecithophyllum anteroporum* Margolis, 1958 (1; 1)
- *Stephanostomum dentatum* (Linton, 1901) (1; 4)
- Cestoda: *Phyllobothrium* sp. (larval) (1; 18)

Gilbertidia sigalutes (Jordan and Starks), soft sculpin (3; 1)
- Nematoda: *Anisakis* sp. (larval) (1; 1)

Hemilepidotus hemilepidotus (Tilesius), red Irish lord (22; 16)
- Digenea: *Anisorchis opisthorchis* Polyansky, 1955 (8; 1–12)
- *Derogenes varicus* (Mueller, 1784) (1; 1)
- *Genolinea laticauda* Manter, 1925 (2; 1)
- *Lecithaster gibbosus* (Rud., 1802) (1; 11)
- *Lepidophyllum cameroni* Arai, 1969 (11; 1–9)
- *Neopodocotyloides sinusaccus* (Ching, 1960) (6; 1–5)
- *Steganoderma formosum* Stafford, 1904 (3; 12–41)
- Nematoda: *Ascarophis sebastodis* Olsen, 1952 (4; 1–9)
- Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (1; 1)

Icelinus filamentosus Gilbert, threadfin sculpin (3; 3)
- Cestoda: *Phyllobothrium* sp. (larval) (1; 1)
- Nematoda: *Contracaecum* sp. (larval) (2; 1)
- Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (1; 1)

Icelinus tenuis Gilbert, spotfin sculpin (2; 2)
- Digenea: Hemiuridae (immature) (1; 1)
- Cestoda: *Phyllobothrium* sp. (larval) (1; 1)

Jordania zonope Starks, longfin sculpin (9; 1)
- Nematoda: *Contracaecum* sp. (larval) (1; 1)

Leptocottus armatus Girard, Pacific staghorn sculpin (10; 10)
- Digenea: Digenea (immature) (1; 4)
- *Genolinea laticauda* Manter, 1925 (1; 5)
- *Neopodocotyloides sinusaccus* (Ching, 1960) (5; 1–9)
- *Podocotyle atomon* (Rud., 1802) (1; 3)
- *Rhipidocotyle elongata* McFarlane, 1936 (1; 4)
- *Tubulovesicula lindbergi* (Layman, 1930) (2; 2)
- Nematoda: *Anisakis* sp. (larval) (1; 1)
- *Contracaecum* sp. (larval) (8; 1–19)

Acanthocephala: *Corynosoma* sp. (juvenile) (1; 1)
Echinorhynchus gadi Mueller, 1776 (3; 1–2)
Malacocottus kincaidi Gilbert and Thompson, blackfin sculpin (4; 3)
Nematoda: *Capillaria* sp. (1; 5)
Contracaecum sp. (larval) (2; 1–2)
Myoxocephalus polyacanthocephalus (Pallas), great sculpin (4; 4)
Digenea: *Genolinea laticauda* Manter, 1925 (4; 2–20)
Podocotyle endophrysi Park, 1937 (1; 47)
Tubulovesicula lindbergi (Layman, 1930) (1; 2)
Cestoda: *Bothriocephalus scorpii* (Mueller, 1776) (4; 1–52)
Phyllobothrium sp. (larval) (1; 2)
Nematoda: *Anisakis* sp. (larval) (1; 4)
Ascarophis sebastodis Olsen, 1952 (2; 5–35)
Contracaecum sp. (larval) (2; 4–9)
Porrocaecum sp. (larval) (2; 1–3)
Acanthocephala: *Corynosoma* sp. (juvenile) (1; 1)
Echinorhynchus gadi Mueller, 1776 (3; 1–10)
Copepoda: *Chondracanthus irregularis* Fraser, 1920 (1; 2)
Lepeophtheirus sp. (1; 1)
Naobranchia occidentalis Wilson, 1915 (1; 1)
Nautichthys oculofasciatus (Girard), sailfin sculpin (6; 6)
Digenea: *Genolinea laticauda* Manter, 1925 (1; 3)
Lecithaster gibbosus (Rud., 1802) (1; 1)
Cestoda: *Bothriocephalus scorpii* (Mueller, 1776) (2; 2)
Nematoda: *Ascarophis sebastodis* Olsen, 1952 (5; 1–9)
Oligocottus maculosus Girard, tidepool sculpin (43; 22)
Digenea: *Genolinea laticauda* Manter, 1925 (3; 1–2)
Lecithaster gibbosus (Rud., 1802) (2; 1)
Tubulovesicula lindbergi (Layman, 1930) (1; 1)
Monogenea: Gyrodactyloidea (3; –)
Nematoda: *Anisakis* sp. (larval) (5; 1)
Contracaecum sp. (larval) (10; 1–9)
Copepoda: *Haemobaphes intermedius* Kabata, 1967 (1; 1)
Haemobaphes sp. (juvenile) (4; 1–6)
Psychrolutes paradoxus Günther, tadpole sculpin (1; 0)
Rhamphocottus richardsoni Günther, grunt sculpin (3; 1)
Nematoda: *Ascarophis sebastodis* Olsen, 1952 (1; 9)
Porrocaecum sp. (larval) (1; 2)
Triglops pingeli Reinhardt, ribbed sculpin (4; 4)
Digenea: *Podocotyle blennicottusi* Park, 1937 (3; 4–8)
Cestoda: *Nybelinia surmenicola* Okada, 1929 (larval) (1; 2)
Phyllobothrium sp. (larval) (2; 1)
Nematoda: *Anisakis* sp. (larval) (2; 1–2)
Contracaecum sp. (larval) (2; 3)
Copepoda: *Haemobaphes* sp. (juvenile) (1; 1)

AGONIDAE

Agonus acipenserinus Tilesius, sturgeon poacher (3; 2)
Digenea: *Podocotyle* sp. (immature) (1; 1)
Cestoda: *Phyllobothrium* sp. (larval) (1; 1)
Nematoda: *Contracaecum aduncum* (Rud., 1802) (larval) (1; 2)
Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (2; 3–5)
Copepoda: *Haemobaphes* sp. (juvenile) (2; –)
Xeneretmus latifrons (Gilbert), blacktip poacher (1; 1)
Cestoda: *Phyllobothrium* sp. (larval) (1; 1)

AMMODYTIDAE

Ammodytes hexapterus Pallas, Pacific sand lance (23; 22)
- Digenea: *Galactosomum* sp. (larval) (9; 1–23)
- *Lecithaster gibbosus* (Rud., 1802) (20; 1–120)
- Monogenea: Gyrodactyloidea (9; –)
- Cestoda: *Phyllobothrium* sp. (larval) (8; 1–16)
- Nematoda: *Anisakis* sp. (larval) (3; 1–2)
- Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (1; 3)
- Copepoda: *Lepeophtheirus* sp. (1; 1)

BATHYMASTERIDAE

Ronquilus jordani (Gilbert), northern ronquil (3; 2)
- Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 8)
- Nematoda: *Anisakis* sp. (larval) (1; 1)
- *Cucullanus* sp. (1; 1)

PHOLIDAE

Apodichthys flavidus Girard, penpoint gunnel (1; 1)
- Nematoda: *Anisakis* sp. (larval) (1; 3)
- *Capillaria* sp. (1; 2)
- Copepoda: *Chondracanthus pusillus* Kabata, 1968 (1; 4)

Pholis laeta (Cope), crescent gunnel (13; 9)
- Digenea: *Podocotyle* sp. (immature) (1; 2)
- Cestoda: *Bothriocephalus* sp. (larval) (2; 1–2)
- Nematoda: *Anisakis* sp. (larval) (3; 1–3)
- *Contracaecum* sp. (larval) (2; 1)
- *Philometra americana* Kuitunen-Ekbaum, 1933 (2; 1)

Pholis ornata (Girard), saddleback gunnel (7; 5)
- Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 2)
- *Neopodocotyloides pedunculata* (Park, 1937) (2; 1–3)
- Monogenea: *Gyrodactyloidea* (2; –)
- Nematoda: *Anisakis* sp. (larval) (3; 5–26)

STICHAEIDAE

Anoplarchus purpurescens Gill, cockscomb (13; 6)
- Nematoda: *Anisakis* sp. (larval) (2; 1–2)
- *Ascarophis sebastodis* Olsen, 1952 (2; 1)
- *Contracaecum* sp. (larval) (1; 1)
- *Porrocaecum* sp. (larval) (1; 2)

Xiphister atropurpureus (Kittlitz), black prickleback (1; 0)

ZOARCIDAE

Aprodon cortezianus Gilbert, bigfin eelpout (5; 4)
- Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 5)
- *Parahemiuris merus* (Linton, 1910) (1; 1)
- *Podocotyle* sp. (immature) (2; 2–29)
- Cestoda: *Bothriocephalus scorpii* (Mueller, 1776) (1; 1)
- *Nybelinia surmenicola* Okada, 1929 (larval) (2; 1–4)
- *Phyllobothrium* sp. (larval) (2; 3–147)
- Nematoda: *Capillaria* sp. (2; –)
- *Contracaecum* sp. (larval) (1; 2)
- Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (1; 2)

PLEURONECTIFORMES

BOTHIDAE

Citharichthys stigmaeus Jordan and Gilbert, speckled sanddab (1; 1)
- Digenea: *Tubulovesicula lindbergi* (Layman, 1930) (1; 1)
- Nematoda: *Anisakis* sp. (larval) (1; 6)

PLEURONECTIDAE

Lepidopsetta bilineata (Ayres), rock sole (6; 6)
- Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 1)
- Cestoda: *Bothriocephalus* sp. (larval) (1; 1)
- *Phyllobothrium* sp. (larval) (2; 1)
- Nematoda: *Anisakis* sp. (larval) (1; 12)
- *Capillaria* sp. (1; 4)
- *Cucullanus* sp. (4; 1–5)
- Copepoda: *Haemobaphes* sp. (juvenile) (1; 1)

Parophrys vetulus Girard, English sole (1; 1)
- Digenea: *Neopodocotyloides sinusaccus* (Ching, 1960) (1; 1)

Platichthys stellatus (Pallas), starry flounder (6; 6)
- Digenea: *Lecithaster gibbosus* (Rud., 1802) (1; 9)
- *Podocotyle atomon* (Rud., 1802) (3; 1–6)
- Monogenea: Gyrodactyloidea (1; –)
- Cestoda: *Phyllobothrium* sp. (larval) (2; 1–3)
- Nematoda: *Anisakis* sp. (larval) (1; 8)
- *Contracaecum* sp. (larval) (2; 3–7)
- *Cucullanus annulatus* Margolis, 1960 (4; 1–3)
- *Philometra* sp. (1; 2)
- Acanthocephala: *Echinorhynchus gadi* Mueller, 1776 (1; 2)
- Copepoda: *Lepeophtheirus* sp. (1; 1)

BATRACHOIDIFORMES

BATRACHOIDIDAE

Porichthys notatus Girard, northern midshipman (2; 1)
- Digenea: *Tubulovesicula lindbergi* (Layman, 1930) (1; 1)

LIST 2. CHECKLIST OF PARASITES WITH HOSTS

DIGENEA

Anisorchis opisthorchis Polyansky, 1955
- *Hemilepidotus hemilepidotus* (Tilesius) (8/22; 1–12); intestine
- *Hexagrammos stelleri* Tilesius (1/3; 11); intestine
- *Hexagrammos superciliosus* (Pallas) (7/31; 1–7); intestine

Crepidostomum farionis (Mueller, 1784)
- *Oncorhynchus kisutsh* (Walbaum) (14/60; 1–23); intestine, pyloric ceca
- *Salvelinus malma* (Walbaum) (1/2; 1); intestine

Deretrema cholaeum McFarlane, 1936
- *Sebastodes maliger* (Jordan and Gilbert) (3/7; 1–2); gall bladder

Derogenes varicus (Mueller, 1784)
- *Gasterosteus aculeatus* Linnaeus (1/23; 3); intestine
- *Hemilepidotus hemilepidotus* (Tilesius) (1/22; 1); intestine

Digenea (immature)
- *Leptocottus armatus* Girard (1/10; 4); intestine
- *Sebastodes caurinus* (Richardson) (2/13; 1); intestine
- *Sebastodes elongatus* (Ayres) (1/3; 2); intestine

Galactosomum sp. (larval)
Ammodytes hexapterus Pallas (9/23; 1–23); gills (encysted)
Cymatogaster aggregata Gibbons (2/138; 1–8); gills (encysted)
Hexagrammos superciliosus (Pallas) (2/31; 1); gills (encysted)
Oncorhynchus keta (Walbaum) (1/34; 2); gills (encysted)
Genolinea laticauda Manter, 1925
Artedius fenestralis Jordan and Gilbert (2/2; 1); stomach
Blepsias cirrhosus (Pallas) (4/6; 1–3); stomach
Hemilepidotus hemilepidotus (Tilesius) (2/22; 1); stomach
Hexagrammos superciliosus (Pallas) (4/31; 1–2); stomach
Leptocottus armatus Girard (1/10; 5); stomach
Myoxocephalus polyacanthocephalus (Pallas) (4/4; 2–20); stomach
Nautichthys oculofasciatus (Girard) (1/6; 3); stomach
Oligocottus maculosus Girard (3/43; 1–2); stomach
Oncorhynchus nerka (Walbaum) (1/8; 1); stomach
Helicometra sp.
Sebastodes maliger (Jordan and Gilbert) (1/7; 2); intestine
Hemiuridae (immature)
Icelinus tenuis Gilbert (1/2; 1); stomach
Lecithaster gibbosus (Rud., 1802)
Ammodytes hexapterus Pallas (20/23; 1–120); intestine
Aprodon cortezianus Gilbert (1/5; 5); intestine
Aulorhynchus flavidus Gill (4/5; 2–50); intestine
Blepsias cirrhosus (Pallas) (1/6; 3); intestine
Brachyistius frenatus Gill (1/4; 2); digestive tract
Clupea harengus pallasi Valenciennes (20/29; 1–22); intestine
Coryphopterus nicholsi (Bean) (1/43; 3); intestine
Cymatogaster aggregata Gibbons (22/138; 1–2); digestive tract
Dasycottus setiger Bean (1/2; 1); intestine
Gadus macrocephalus Tilesius (18/18; 3–51); intestine, pyloric ceca
Gasterosteus aculeatus Linnaeus (5/23; 1–4); intestine
Hemilepidotus hemilepidotus (Tilesius) (1/22; 11); intestine
Hexagrammos stelleri Tilesius (3/3; 1–5); intestine
Hexagrammos superciliosus (Pallas) (15/31; 1–17); intestine
Lepidopsetta bilineata (Ayres) (1/6; 1); intestine
Nautichthys oculofasciatus (Girard) (1/6; 1); intestine
Oligocottus maculosus Girard (2/43; 1); intestine
Oncorhynchus gorbuscha (Walbaum) (18/81; 1–9); intestine
Oncorhynchus keta (Walbaum) (16/34; 1–15); intestine, pyloric ceca
Oncorhynchus kisutch (Walbaum) (27/60; 1–40); intestine, pyloric ceca
Oncorhynchus nerka (Walbaum) (7/8; 4–43); intestine
Oncorhynchus tshawytscha (Walbaum) (1/1; 28); intestine
Ophiodon elongatus Girard (27/34; 1–52); intestine
Pholis ornata (Girard) (1/7; 2); intestine
Platichthys stellatus (Pallas) (1/6; 9); intestine
Ronquilus jordani (Gilbert) (1/3; 8); intestine
Salvelinus malma (Walbaum) (2/2; 10–21); intestine, pyloric ceca
Syngnathus griseolineatus Ayres (8/25; 1–2); intestine
Thaleichthys pacificus (Richardson) (1/7; 2); intestine
Theragra chalcogramma (Pallas) (1/2; 6); intestine
Lecithophyllum anteroporum Margolis, 1958
Dasycottus setiger Bean (1/2; 1); intestine
Hexagrammos stelleri Tilesius (1/3; 1); intestine
Hexagrammos superciliosus (Pallas) (1/31; 1); intestine

Lepidapedon microcotyleum Dollfus, 1953
Theragra chalcogramma (Pallas) (1/2; 1); intestine
Lepidophyllum cameroni Arai, 1969
Hemilepidotus hemilepidotus (Tilesius) (11/22; 1–9); urinary bladder
Neopodocotyloides pedunculata (Park, 1937)
Pholis ornata (Girard) (2/7; 1–3); intestine
Neopodocotyloides sinusaccus (Ching, 1960)
Blepsias cirrhosus (Pallas) (4/6; 1–4); intestine
Gasterosteus aculeatus Linnaeus (1/23; 1); intestine
Hemilepidotus hemilepidotus (Tilesius) (6/22; 1–5); intestine
Hexagrammos stelleri Tilesius (1/3; 4); intestine
Leptocottus armatus Girard (5/10; 1–9); intestine
Parophrys vetulus Girard (1/1; 1); intestine
Sebastodes maliger (Jordan and Gilbert) (1/7; 2); intestine
Syngnathus griseolineatus Ayres (1/25; 2); intestine
Theragra chalcogramma (Pallas) (1/2; 15); intestine
Neozoogonus californicus Arai, 1954
Cymatogaster aggregata Gibbons (21/138; 1–80); digestive tract
Opechona occidentalis Montgomery, 1957
Sebastodes caurinus (Richardson) (6/13; 1–18); intestine
Sebastodes elongatus (Ayres) (1/3; 1); intestine
Syngnathus griseolineatus Ayres (1/25; 1); intestine
Parahemiuris merus (Linton, 1910)
Aprodon cortezianus Gilbert (1/5; 1); intestine
Clupea harengus pallasi Valenciennes (3/20; 2–5); intestine, stomach
Gadus macrocephalus Tilesius (8/18; 1–6); intestine, stomach, pyloric ceca
Hexagrammos stelleri Tilesius (1/3; 3); intestine
Oncorhynchus kisutch (Walbaum) (5/60; 1–8); intestine, pyloric ceca
Oncorhynchus nerka (Walbaum) (1/8; 4); intestine
Ophiodon elongatus Girard (2/34; 1); intestine
Syngnathus griseolineatus Ayres (1/25; 1); intestine
Pentagramma petrowi (Layman, 1930)
Clupea harengus pallasi Valenciennes (3/29; 1–3); intestine
Podocotyle atomon (Rud., 1802)
Aulorhynchus flavidus Gill (1/5; 8); intestine
Leptocottus armatus Girard (1/10; 3); intestine
Platichthys stellatus (Pallas) (3/6; 1–6); intestine
Podocotyle blennicottusi Park, 1937
Triglops pingeli Reinhardt (3/4; 4–8); intestine
Podocotyle endophrysi Park, 1937
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 47); intestine, pyloric ceca
Syngnathus griseolineatus Ayres (4/25; 1–2); intestine
Podocotyle sp. (immature)
Agonus acipenserinus Tilesius (1/3; 1); intestine
Aprodon cortezianus Gilbert (2/5; 2–29); intestine
Blepsias cirrhosus (Pallas) (1/6; 1); intestine
Hexagrammos stelleri Tilesius (1/3; 3); intestine
Pholis laeta (Cope) (1/13; 2); intestine
Sebastodes alutus (Gilbert) (2/3; 3–5); intestine
Rhipidocotyle elongata McFarlane, 1936
Leptocottus armatus Girard (1/10; 4); intestine
Ophiodon elongatus Girard (1/34; 6); intestine
Steganoderma formosum Stafford, 1904
Hemilepidotus hemilepidotus (Tilesius) (3/22; 12–41); intestine

Stephanostomum casum (Linton, 1910)
Artedius harringtoni Starks (1/11; 1); intestine
Sebastodes caurinus (Richardson) (3/13; 1–9); intestine
Stephanostomum dentatum (Linton, 1901)
Dasycottus setiger Bean (1/2; 4); intestine
Stephanostomum tristephanum McFarlane, 1936
Ophiodon elongatus Girard (1/34; 8); intestine
Telolecithus pugetensis Lloyd and Guberlet, 1932
Cymatogaster aggregata Gibbons (20/138; 1–100); digestive tract
Tubulovesicula lindbergi (Layman, 1930)
Citharichthys stigmaeus Jordan and Gilbert (1/1; 1); stomach
Cymatogaster aggregata Gibbons (1/138; 2); digestive tract
Leptocottus armatus Girard (2/10; 2); stomach
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 2); stomach
Oligocottus maculosus Girard (1/43; 1); stomach
Ophiodon elongatus Girard (2/34; 1–52); stomach
Porichthys notatus Girard (1/1; 1); stomach
Salvelinus malma (Walbaum) (2/2; 1–2); stomach
Syngnathus griseolineatus Ayres (2/25; 1); stomach

MONOGENEA

Gyrodactyloidea
Ammodytes hexapterus Pallas (9/25; –); gills
Clupea harengus pallasi Valenciennes (9/29; –); gills, body surface
Cymatogaster aggregata Gibbons (29/138; –); gills, body surface
Oligocottus maculosus Girard (3/43; –); gills
Oncorhynchus kisutch (Walbaum) (1/60; 1); gills
Pholis ornata (Girard) (2/7; –); gills
Platichthys stellatus (Pallas) (1/6; –); gills
Syngnathus griseolineatus Ayres (1/25; –); gills
Megalocotyle marginata Folda, 1928
Sebastodes caurinus (Richardson) (2/13; 2–4); gills
Microcotyle sebastis Goto, 1894
Hexagrammos superciliosus (Pallas) (3/31; 2–9); gills
Sebastodes caurinus (Richardson) (5/13; 1–6); gills
Sebastodes elongatus (Ayres) (2/3; 6–12); gills

CESTODA

Bothriocephalus scorpii (Mueller, 1776)
Artedius harringtoni Starks (6/11; 1–2); intestine
Myoxocephalus polyacanthocephalus (Pallas) (4/4; 1–52); intestine
Nautichthys oculofasciatus (Girard) (2/6; 2); intestine
Bothriocephalus scorpii (Mueller, 1776) (larval)
Aprodon cortezianus Gilbert (1/5; 1); intestine
Gasterosteus aculeatus Linnaeus (3/23; 2–4); intestine
Hexagrammos superciliosus (Pallas) (1/31; 1); intestine
Theragra chalcogramma (Pallas) (2/2; 2–3); intestine
Bothriocephalus sp. (larval)
Aulorhynchus flavidus Gill (1/5; 2); intestine
Lepidopsetta bilineata (Ayres) (1/6; 1); intestine
Pholis laeta (Cope) (2/13; 1–2); intestine
Sebastodes alutus (Gilbert) (1/3; 1); intestine

Diphyllobothrium sp. (larval)
Oncorhynchus kisutch (Walbaum) (9/60; 1–2); body cavity
Oncorhynchus nerka (Walbaum) (7/8; 1–3); body cavity
Nybelinia surmenicola Okada, 1929 (larval)
Aprodon cortezianus Gilbert (2/5; 1–4); intestine
Ophiodon elongatus Girard (1/34; 115); intestine, stomach
Theragra chalcogramma (Pallas) (1/2; 26); intestine, stomach
Triglops pingeli Reinhardt (1/4; 2); intestine
Phyllobothrium sp. (larval)
Agonus acipenserinus Tilesius (1/3; 1); intestine
Ammodytes hexapterus Pallas (8/23; 1–16); intestine
Aprodon cortezianus Gilbert (2/5; 3–147); intestine
Aulorhynchus flavidus Gill (1/5; 1); intestine
Blepsias cirrhosus (Pallas) (2/6; 1); intestine
Clupea harengus pallasi Valenciennes (10/29; 1–2); intestine
Coryphopterus nicholsi (Bean) (1/43; 3); intestine
Cymatogaster aggregata Gibbons (5/138; 1–3); digestive tract
Dasycottus setiger Bean (1/2; 18); intestine
Gadus macrocephalus Tilesius (5/18; 1–2); intestine
Gasterosteus aculeatus Linnaeus (8/23; 1–7); intestine
Hexagrammos stelleri Tilesius (1/3; 3); intestine
Hexagrammos superciliosus (Pallas) (21/31; 2–32); intestine
Icelinus filamentosus Gilbert (1/3; 1); intestine
Icelinus tenuis Gilbert (1/2; 1); intestine
Lepidopsetta bilineata (Ayres) (2/6; 1); intestine
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 2); intestine
Ocorhynchus gorbuscha (Walbaum) (10/81; 1–2); intestine
Oncorhynchus keta (Walbaum) (2/34; 1); intestine
Oncorhynchus kisutch (Walbaum) (10/60; 1–2); intestine
Oncorhynchus nerka (Walbaum) (3/8; 2–4); intestine
Oncorhynchus tshawytscha (Walbaum) (1/1; 1); intestine
Ophiodon elongatus Girard (14/34; 1–2); intestine
Platichthys stellatus (Pallas) (2/6; 1–3); intestine
Salvelinus malma (Walbaum) (1/2; 1); intestine
Sebastodes diploprora (Gilbert) (1/1; 3); intestine
Sebastodes maliger (Jordan and Gilbert) (1/7; 1); intestine
Syngnathus griseolineatus Ayres (1/25; 1); intestine
Thaleichthys pacificus (Richardson) (1/7; 1); intestine
Theragra chalcogramma (Pallas) (1/2; 1); intestine
Triglops pingeli Reinhardt (2/4; 1); intestine
Xeneretmus latifrons (Gilbert) (1/1; 1); intestine
Proteocephalus sp. (larval)
Oncorhynchus kisutch (Walbaum) (2/60; 1); intestine

NEMATODA

Anisakis sp. (larval)
Ammodytes hexapterus Pallas (3/23; 1–2); body cavity
Anoplarchus purpurescens Gill (2/13; 1–2); body cavity
Apodichthys flavidus Girard (1/1; 3); body cavity
Aulorhynchus flavidus Gill (2/5; 1–2); body cavity
Blepsias cirrhosus (Pallas) (2/6; 1); body cavity
Citharichthys stigmaeus Jordan and Gilbert (1/1; 6); body cavity
Clupea harengus pallasi Valenciennes (4/29; 2–17); body cavity

Gadus macrocephalus Tilesius (1/18; 1); body cavity
Gasterosteus aculeatus Linnaeus (4/23; 1); body cavity
Gilbertidia sigalutes (Jordan and Starks) (1/3; 1); body cavity
Hexagrammos superciliosus (Pallas) (7/31; 1–2); body cavity
Lepidopsetta bilineata (Ayres) (1/6; 12); body cavity
Leptocottus armatus Girard (1/10; 1); body cavity
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 4); intestine
Oligocottus maculosus Girard (5/43; 1); body cavity
Oncorhynchus gorbuscha (Walbaum) (2/81; 1); body cavity
Oncorhynchus keta (Walbaum) (2/34; 1–2); body cavity
Oncorhynchus kisutch (Walbaum) (5/60; 1–5); body cavity
Oncorhynchus nerka (Walbaum) (2/8; 1); body cavity
Ophiodon elongatus Girard (1/34; 315); stomach
Pholis laeta (Cope) (3/13; 1–3); body cavity
Pholis ornata (Girard) (3/7; 5–26); body cavity
Platichthys stellatus (Pallas) (1/6; 8); intestine
Ronquilus jordani (Gilbert) (1/3; 1); body cavity
Sebastodes alutus (Gilbert) (1/3; 1); body cavity
Syngnathus griseolineatus Ayres (4/36; 1–3); body cavity
Theragra chalcogramma (Pallas) (1/2; 1); body cavity
Triglops pingeli Reinhardt (2/4; 1–2); body cavity
Ascarophis sebastodis Olsen, 1952
Anoplarchus purpurescens Gill (2/13; 1); intestine
Artedius harringtoni Starks (1/11; 22); intestine
Aulorhynchus flavidus Gill (1/5; 1); intestine
Hemilepidotus hemilepidotus (Tilesius) (4/22; 1–9); intestine
Hexagrammos superciliosus (Pallas) (1/31; 4); intestine
Myoxocephalus polyacanthocephalus (Pallas) (2/4; 5(35); intestine
Nautichthys oculofasciatus (Girard) (5/6; 1–9); intestine
Oncorhynchus kisutch (Walbaum) (7/60; 1–7); intestine
Oncorhynchus nerka (Walbaum) (1/8; 1); intestine
Rhamphocottus richardsoni Günther (1/3; 9); intestine
Sebastodes caurinus (Richardson) (11/13; 1–120); intestine
Sebastodes elongatus (Ayres) (1/3; 2); intestine
Capillaria sp.
Apodichthys flavidus Girard (1/1; 2); stomach
Aprodon cortezianus Gilbert (2/5; –); stomach
Chitonotus pugetensis (Steindachner) (1/2; 3); stomach
Lepidopsetta bilineata (Ayres) (1/6; 4); stomach
Malacocottus kincaidi (Ayres) (1/6; 4); stomach
Oncorhynchus kisutch (Walbaum) (2/60; 1–3); stomach
Oncorhynchus tshawytscha (Walbaum) (1/1; 2); stomach
Contracaecum aduncum (Rud., 1802) (larval)
Agonus acipenserinus Tilesius (1/3; 2); intestine
Aulorhynchus flavidus Gill (2/5; 8–12); body cavity
Contracaecum sp. (larval)
Anoplarchus purpurescens Gill (1/31; 1); body cavity
Aprodon cortezianus Gilbert (1/5; 2); body cavity
Artedius harringtoni Starks (3/11; 1); body cavity
Chitonotus pugetensis (Steindachner) (2/2; 3); body cavity
Clupea harengus pallasi Valenciennes (3/29; 1–10); body cavity
Coryphopterus nicholsi (Bean) (30/43; 1–3); body cavity
Cymatogaster aggregata Gibbons (1/138; 2); body cavity
Hexagrammos superciliosus (Pallas) (16/31; 1–7); body cavity, stomach

Icelinus filamentosus Gilbert (2/3; 1); body cavity
Jordania zonope Starks (1/9; 1); body cavity
Leptocottus armatus Girard (8/10; 1–19); body cavity
Malacocottus kincaidi Gilbert and Thompson (2/4; 1–2); body cavity
Myoxocephalus polyacanthocephalus (Pallas) (2/4; 4–9); body cavity, intestine
Oligocottus maculosus Girard (10/43; 1–9); body cavity
Oncorhynchus gorbuscha (Walbaum) (2/81; 1); body cavity
Oncorhynchus keta (Walbaum) (3/34; 1–7); body cavity
Oncorhynchus kisutch (Walbaum) (2/60; 1–2); body cavity
Oncorhynchus nerka (Walbaum) (2/8; 1); body cavity
Oncorhynchus tshawytscha (Walbaum) (1/1; 3); body cavity
Pholis laeta (Cope) (2/13; 1); body cavity
Platichthys stellatus (Pallas) (2/6; 3–7); body cavity
Salvelinus malma (Walbaum) (1/2; 1); body cavity
Sebastodes caurinus (Richardson) (6/13; 1–2); body cavity
Syngnathus griseolineatus Ayres (2/36; 1); body cavity
Thaleichthys pacificus (Richardson) (1/7; 1); body cavity
Theragra chalcogramma (Pallas) (1/2; 13); intestine
Triglops pingeli Reinhardt (2/4; 3); body cavity
Cucullanus annulatus Margolis, 1960
Platichthys stellatus (Pallas) (4/6; 1–3); intestine
Cucullanus smedleyi Campana-Rouget, 1957
Sebastodes caurinus (Richardson) (4/14; 1); intestine
Cucullanus sp.
Cymatogaster aggregata Gibbons (42/138; 1–11); digestive tract
Lepidopsetta bilineata (Ayres) (1/6; 5); intestine
Oncorhynchus kisutch (Walbaum) (1/60; 1); intestine
Ronquilus jordani (Gilbert) (1/3; 1); intestine
Philometra americana Kuitunen-Ekbaum, 1933
Pholis laeta (Cope) (2/13; 1); intestine
Philometra sp.
Platichthys stellatus (Pallas) (1/6; 2); intestine
Philonema sp. (larval)
Oncorhynchus kisutch (Walbaum) (10/60; –); swim bladder wall
Oncorhynchus tshawytscha (Walbaum) (1/1; 2); swim bladder wall
Porrocaecum sp. (larval)
Anoplarchus purpurescens Gill (1/13; 2); body cavity
Myoxocephalus polyacanthocephalus (Pallas) (2/4; 1–3); intestine
Ophiodon elongatus Girard (3/34; 1–3); body cavity, stomach
Rhamphocottus richardsoni Günther (1/3; 2); body cavity
Theragra chalcogramma (Pallas) (1/2; 14); intestine

ACANTHOCEPHALA

Corynosoma sp. (juvenile)
Cymatogaster aggregata Gibbons (1/138; 1); body cavity
Leptocottus armatus Girard (1/10; 1); intestine
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 1); intestine
Oncorhynchus kisutch (Walbaum) (1/60; 1); intestine
Echinorhynchus gadi Mueller, 1776
Agonus acipenserinus Tilesius (2/3; 3–5); intestine
Ammodytes hexapterus Pallas (1/23; 3); intestine
Aprodon cortezianus Gilbert (1/5; 2); intestine
Cymatogaster aggregata Gibbons (1/138; 1); intestine

Hemilepidotus hemilepidotus (Tilesius) (1/22; 1); intestine
Icelinus filamentosus Gilbert (1/3; 1); intestine
Leptocottus armatus Girard (3/10; 1–2); intestine
Myoxocephalus polyacanthocephalus (Pallas) (3/4; 1–10); intestine
Oncorhynchus kisutch (Walbaum) (1/60; 1); intestine
Oncorhynchus tshawytscha (Walbaum) (1/1; 1); intestine
Platichthys stellatus (Pallas) (1/6; 2); intestine
Salvelinus malma (Walbaum) (1/2; 6); intestine
Neoechinorhynchus rutili (Mueller, 1780)
Oncorhynchus keta (Walbaum) (1/34; 1); intestine
Oncorhynchus kisutch (Walbaum) (4/60; 1–6); intestine
Oncorhynchus nerka (Walbaum) (3/8; 1–5); intestine

COPEPODA

Caligus clemensi Parker and Margolis, 1964
Clupea harengus pallasi Valenciennes (2/29; 1); body surface
Chondracanthus irregularis Fraser, 1920
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 2); gills
Chondracanthus pusillus Kabata, 1968
Apodichthys flavidus Girard (1/1; 4); gills
Ergasilus auritus Markevich, 1940
Gasterosteus aculeatus Linnaeus (22/23; 4–20); gills, body surface
Ergasilus turgidus Fraser, 1920
Cymatogaster aggregata Gibbons (61/138; 1–105); gills
Haemobaphes diceraus Wilson, 1917
Cymatogaster aggregata Gibbons (13/138; 1–3); gills
Haemobaphes intermedius Kabata, 1967
Oligocottus maculosus Girard (1/43; 1); gills
Haemobaphes sp. (juvenile)
Agonus acipenserinus Tilesius (2/3; –); gills
Blepsias cirrhosus (Pallas) (2/6; 2); gills
Hexagrammos decagrammus (Pallas) (1/1; 14); gills
Lepidopsetta bilineata (Ayres) (1/6; 1); gills
Oligocottus maculosus Girard (4/43; 1–6); gills
Syngnathus griseolineatus Ayres (8/36; 1); gills
Triglops pingeli Reinhardt (1/4; 1); gills
Lepeophtheirus sp.
Ammodytes hexapterus Pallas (1/23; 1); body surface
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 1); body surface
Platichthys stellatus (Pallas) (1/6; 1); body surface
Naobranchia occidentalis Wilson, 1915
Myoxocephalus polyacanthocephalus (Pallas) (1/4; 1); gills
Parabomolochus cuneatus (Fraser, 1920)
Clupea harengus pallasi Valenciennes (1/29; 2); gills
Cymatogaster aggregata Gibbons (30/138; 1–4); gills
Salmincola californiensis Dana, 1852
Oncorhynchus kisutch (Walbaum) (1/60; 1); pectoral fin

ISOPODA

Livoneca californica Schioedte and Meinert, 1884
Cymatogaster aggregata Gibbons (11/138; 1); gill chamber

DISCUSSION

According to Boyce (1969) *L. gibbosus* (Rud., 1802) and larval *Phyllobothrium* were the two parasites that showed the highest incidences of infection in young pink salmon examined from Burke Channel. My findings corroborate his results on *O. gorbuscha* and, in addition, show that these parasites are those most frequently encountered in a variety of other host species. From this information it is possible to conclude that resident fishes are responsible for the perpetuation of these parasites in Burke Channel, acting as reservoirs from which the pink salmon acquire the parasites during their migration to the open sea. The three other parasites reported by Boyce, *Parahemiuris merus* Linton, 1910, *Pentagramma petrowi* (Layman, 1930), and *Contracaecum* sp., although encountered much less frequently, probably assume a comparable pattern in their host relations.

Aside from the considerations above, two main points become apparent when the results of this study are examined; these are (1) that certain parasites appear to have a wide range of hosts, i.e., are not host specific; and (2) that fishes occupying the same or similar niches tend to harbour parasites that are common to many of them. However, before considering either of these points, it should be emphasized that forms allocated to a number of the taxa could represent more than one species. This consideration is especially important for forms referred to *Phyllobothrium*, *Cucullanus*, *Contracaecum*, and Gyrodactyloidea.

Since its specific identity has been established and since it is one of the species most frequently recovered in this study, the best example to use in discussing the two points above is *L. gibbosus*. This intestinal trematode was recovered from 30 host species, of which 228 of 677 (33.7%) individuals harboured the parasite. The data show that pelagic fishes commonly taken in purse seines with pink salmon are those most frequently parasitized by *L. gibbosus*. Juvenile *Gadus macrocephalus* (100%), *Ammodytes hexapterus* (87%), juvenile *Ophiodon elongatus* (79%), and *Clupea harengus pallasi* (69%) were host species that showed the higher levels of infection. These results indicate clearly that fishes that share a common niche and have similar feeding habits harbour parasites that are common to them. In addition, it would be interesting to speculate as to how fishes that are usually considered benthic and sedentary acquire infections with this trematode; however, the feeding habits as well as other aspects of the biology of these hosts are not well known so that further comment is not justified.

Reports in the literature frequently allude to the importance of gyrodactyloid Monogenea in contributing to mortality in fishes. Members of this group of ectoparasites were collected from individuals of nine host species. However, no detrimental effects were observed in the field-caught parasitized fishes and none of these parasites was recovered from *O. gorbuscha*. On the other hand, when samples of *C. harengus pallasi* or *Gasterosteus aculeatus* were maintained in aquaria to determine whether interspecific cross-infections

could occur with pink salmon, light to moderate mortality was recorded within 2 months in the natural hosts. Examinations of the dead or dying fishes showed that the intensity of infection with ectoparasitic flukes was much greater than that recorded for hosts examined immediately after capture. The inference that these parasites contributed to mortality is further supported when examinations of the surviving fishes showed that the extent of parasitism was much less in the latter instances. However, the possibility that weaker fish were more susceptible to parasitism cannot be ignored. In the cross-infection experiments, only one specimen of pink salmon became infected with a gyrodactyloid, and in this instance with only a single parasite.

The only example in the entire series of examinations in which there was any evidence of possible detrimental effects of parasites was that of a lingcod (*Ophiodon elongatus*). This fish was a large (excess of 3 ft in length) adult female that harboured five species of parasites; of these, nematodes of the genus *Anisakis* were found in three ulcerous cavities. These cavities, located in the stomach wall, were nearly 3 cm in diameter. The wall was not perforated, but only the outer connective tissue layer remained intact. No weights nor precise measurements of the fish were taken; however, comparisons with other specimens collected in the past indicate that the fish was very emaciated and appeared to weigh about half the usual for a fish of comparable length in "healthy condition."

ACKNOWLEDGMENTS

I am indebted to Drs Robert Parker and Leo Margolis for their efforts and cooperation which made this work possible. I am also indebted to various members of their respective investigations for their assistance in the collection of host animals and in providing information that proved invaluable in carrying out the investigation to this point of completion. I am grateful for the assistance of Drs Z. Kabata and L. S. Roberts who identified the Copepoda and also of Mr J. Thomson who identified the species of *Sebastodes*. I am thankful for the office facilities furnished by Dr Clark P. Read, Rice University, during the completion of the manuscript.

REFERENCES

ARAI, HISAO P. 1967. Ecological specificity of parasites of some embiotocid fishes. J. Fish. Res. Bd. Canada 24: 2161–2168.

BAILEY, R. M., E. A. LACHNER, C. C. LINDSAY, C. R. ROBINS, P. M. ROEDEL, W. B. SCOTT, AND L. P. WOOD. 1960. A list of common and scientific names of fishes from the United States and Canada. 2nd ed. Am. Fish. Soc. Spec. Publ. 2. 102 p.

BOYCE, N. P. J. 1969. Parasite fauna of pink salmon (*Oncorhynchus gorbuscha*) of the Bella Coola River, central British Columbia, during their early sea life. J. Fish. Res. Bd. Canada 26: 813–820.

CLEMENS, W. A., AND G. V. WILBY. 1961. Fishes of the Pacific Coast of Canada. 2nd ed. Bull. Fish. Res. Bd. Canada 68. 443 p.

YAMAGUTI, S. 1958. Systema Helminthum. Vol. I. The digenetic trematodes of vertebrates. Pt. I and II. Interscience Publishers, Inc., New York and London. 859 p.

1959. Systema Helminthum. Vol. II. The cestodes of vertebrates. Interscience Publishers, Inc., New York and London. 859 p.

1961. Systema Helminthum. Vol. III. The nematodes of vertebrates. Pt. I and II. Interscience Publishers, Inc., New York and London. 1261 p.

1963a. Systema Helminthum. Vol. IV. Monogenea and Aspidocotylea. Interscience Publishers, Inc., New York and London. 669 p.

1963b. Systema Helminthum. Vol. V. Acanthocephala. Interscience Publishers, Inc., New. York and London. 423 p.

Relationships of Female Age and Size to Embryo Number and Size in the Shiner Perch, *Cymatogaster aggregata*[1]

DENNIS C. WILSON AND RAYMOND E. MILLEMANN

Department of Fisheries and Wildlife
Oregon State University, Marine Science Center, Newport, Ore. 97365, USA

WILSON, DENNIS C., AND RAYMOND E. MILLEMANN. 1969. Relationships of female age and size to embryo number and size in the shiner perch, *Cymatogaster aggregata*. J. Fish. Res. Bd. Canada 26: 2339–2344.

Ages, lengths, and weights were determined for 124 female shiner perch, *Cymatogaster aggregata* Gibbons, collected in Yaquina Bay, Oregon, from May 17 through June 29, 1968. Embryos (1005) were obtained from 111 gravid females either by dissection (737) or at parturition in the laboratory (268). Embryo lengths, weights, and numbers per female parent were determined.

As females increased in age from 1 to 6 years, their mean fork length increased from 9.32 to 13.65 cm, their weight from 13.65 to 47.78 g, and the mean number of embryos per parent female increased from 5.83 to 20.00. The equations and their correlation coefficients (R) computed for the relationships of embryo number (Y) to parent female fork length, weight, and age are, respectively: $\log Y = -1.892 + 2.735 \log X$ (R = 0.89); $Y = 1.131 + 0.313 X$ (R = 0.70); and $Y = 4.23 + 1.73 X$ (R = 0.51). Embryo size at birth was also directly related to female parent size. The equations computed for the relationships of total length of embryo at birth (Y) to parent female fork length and parent female weight are, respectively: $Y = 24.211 + 1.620 X$ (R = 0.79); and $Y = 0.384 + 0.015 X$ (R = 0.72). The equation computed for the relationship of embryo weight (Y) to embryo length at birth is $Y = -2.266 + 0.712 X$ (R = 0.95).

Received April 8, 1969

INTRODUCTION

THE VIVIPAROUS SHINER PERCH, *Cymatogaster aggregata* Gibbons, is distributed along the Pacific coast from southern California to Alaska. These fish inhabit the bays and estuaries in large numbers during the summer months, and at this time breeding and parturition occur. For these reasons, we chose the shiner perch as a test animal for use in studies on the effects of pesticides, which may enter estuaries, on growth and reproduction of representative estuarine organisms. However, before beginning these studies we needed to assess the natural variation of the relationships between female size and embryo size and number. This information, which is the subject of the present paper, is not available in sufficient detail from the studies of Suomela (MS, 1931) and Gordon (MS, 1965) on age and growth of *C. aggregata* or from the reports of Wiebe (1968a, b), who studied the reproductive cycle of shiner perch and

[1]Technical Paper 2649, Oregon Agricultural Experiment Station.

Printed in Canada (J1436)

the effects of temperature and daylight on the reproductive physiology. Data similar to ours have been reported for three other embiotocid species, namely, the barred surfperch (Hubbs, 1921; Carlisle et al., 1960), the striped perch (Swedberg, MS, 1965), and the pile perch (Wares, MS, 1968).

MATERIALS AND METHODS

Four samples consisting of 100 adult female shiner perch were collected in Yaquina Bay, Oregon, at 2-week intervals from May 17, 1968, through June 29, 1968. The weight of each female less that of its embryos was recorded to the nearest 0.05 g and the fork length to the nearest 0.1 cm. Ages of the fish were determined by examination of scales. Scales from a key area under the left lateral line of each fish were removed, pressed on acetate cards, and read separately by three persons.

Embryos were removed individually from the female uterus and counted. Their weights to the nearest 0.001 g and total lengths to the nearest 0.01 cm were recorded. Total length was used because the caudal fin fork does not develop until just before birth of the embryo.

The relationship between female size and size of embryos at birth was determined from an additional sample of 24 fish collected in Yaquina Bay, Oregon, on July 1, 1968. These fish were held in the laboratory in 12-liter glass jars with continuously flowing sea water of about 30‰ salinity and at about 12.5 C. The fish were fed minced cockle clams, *Clinocardium nuttalli*, daily. At the time of parturition, which was 3–14 days after the females were collected, the young fish were removed from the jars and their weights and total lengths recorded, as well as those of a few removed from the females by dissection. Weights and fork lengths of the parent females also were recorded.

Standard length (SL) and total length (TL) were recorded for each of 71 females, which were 5.4–13.4 cm in fork length (FL). We computed equations from these measurements for conversion of FL to SL or TL, so that we could compare our results with those of others. These equations are

$$TL = 0.54 + 1.05\ FL$$

and

$$SL = -0.34 + 0.92\ FL.$$

The respective correlation coefficients (R) are 0.96 and 0.97.

The age, length, and weight data were compiled on computer cards using a separate card for each embryo. A linear regression analysis program was used to obtain the equations for the various relationships. Logarithmic transformation was used to compute the equation for one relationship that did not prove linear when arithmetic coordinates were used.

RESULTS

Age Group Relationships

The 121 female fish, of which 109 were gravid, were 1–6 years old; 108 of the 121 females were 1–3 years old (Table 1). As females increased in age from 1 to 6 years, their mean fork length increased from 9.32 to 13.65 cm, their mean weight from 13.65 to 47.78 g, and the mean number of embryos per female increased from 5.83 to 20.00. The equations computed for the relationships of embryo number (Y) to parent female fork length (Fig. 1), weight (Fig. 2), and age are, respectively:

$$\log Y = -1.892 + 2.735 \log X\ (R = 0.89);$$

$$Y = 1.131 + 0.313\ X\ (R = 0.70);$$

and

$$Y = 4.23 + 1.73\ X\ (R = 0.51).$$

TABLE 1. Mean lengths and weights of female shiner perch, *Cymatogaster aggregata*, and the mean number of embryos per female fish for each age class.

	Female fish[a]			
Age-group	No. examined/no. gravid	Mean fork length (*cm*)	Mean wt (g)	Mean no. embryos per female
I	51/46	9.32±0.59[b]	13.65±2.64	5.83±0.86
II	32/28	11.11±0.54	24.12±3.55	9.30±1.69
III	25/24	11.78±0.61	28.60±4.50	11.10±1.89
IV	9/8	12.96±0.84	37.09±6.05	15.40±4.33
V	2/1	13.35	44.95	15.00
VI	2/2	13.65	47.78	20.00
Total	121/109			

[a]Scales of three of the 124 fish collected were not readable.
[b]Standard deviation of mean.

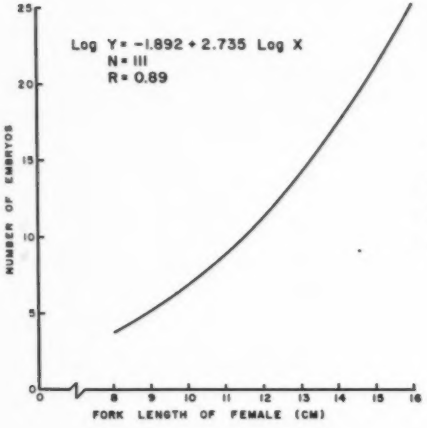

FIG. 1. Regression of the number of *Cymatogaster aggregata* embryos compared to fork length of parent females. (Points in this and succeeding figures not shown for reason of clarity.)

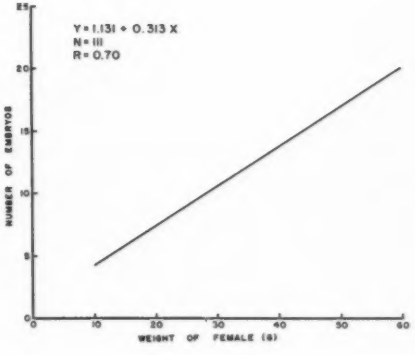

FIG. 2. Regression of the number of *Cymatogaster aggregata* embryos compared to weight of parent females.

The number of embryos per female ranged from 3 to 25. Yearling and 2-year-old females can be recognized by their size and number of embryos per female, but with fish in age-groups II through VI there is some overlap of the relationships between these characteristics and age (Table 1).

Sampling Time Relationships

The number of embryos measured was 1005; these were obtained from 111 gravid females. The mean total length and mean weight of embryos collected on May 17 and June 29 were 2.23 cm and 0.102 g and 3.62 cm and 0.484 g, respectively; and the mean total length and mean weight of embryos at parturition were 4.37 cm and 0.842 g (Table 2). The sample of newborn young was a highly selective one and this explains the large difference in mean size between these fish and the embryos obtained in the field only 1–2 weeks earlier.

Table 2. Mean lengths and weights of female and embryo shiner perch, *Cymatogaster aggregata*, for each sampling period.

Sample date (*1968*)	Adults			Embryos		
	No. examined /no. gravid	Mean fork length (*cm*)	Mean wt (*g*)	Sample size	Mean total length (*cm*)	Mean wt (*g*)
May 17	28/25	11.33±1.55[a]	26.95±11.45	255	2.23±0.61	0.102±0.085
June 2	27/23	10.62±1.50	21.36±9.38	174	2.73±0.59	0.173±0.077
June 16	25/25	11.09±1.14	24.66±7.39	216	3.77±0.37	0.474±0.145
June 29	20/14	10.08±0.96	18.04±6.11	92	3.62±1.02	0.484±0.222
July 4–15[b]	24/24	12.01±1.11	30.39±8.16	268	4.37±0.23	0.842±0.171
Total	124/111			1005		

[a]Standard deviation of the mean.
[b]These fish were collected July 1, 1968, and held in the laboratory to the time of parturition.

Size of embryos at birth was directly related to female parent size (Fig. 3, 4). The equations computed for the relationships of total length of embryo at birth (Y) to parent female fork length and parent female weight are, respectively:

$$Y = 24.211 + 1.620\,X \quad (R = 0.79);$$

and

$$Y = 0.384 + 0.015\,X \quad (R = 0.72).$$

Size of embryos collected in the field was also directly related to female parent size for each sample date. The smallest embryo, from a parent 9.7 cm long and 14.4 g in weight, was 3.90 cm long and 0.454 g in weight at birth; the largest embryo, from a parent 14.0 cm long and 40.0 g in weight, was 4.90 cm long and 1.256 g in weight at birth. The equation computed for the relationship of embryo weight (Y) to embryo length at birth (Fig. 5) is

$$Y = -2.266 + 0.712\,X \quad (R = 0.95).$$

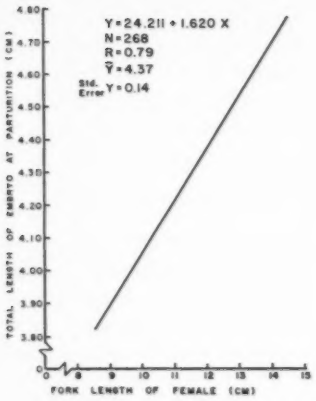

FIG. 3. Regression of the total length of *Cymatogaster aggregata* embryos at parturition compared to the fork length of 24 parent females.

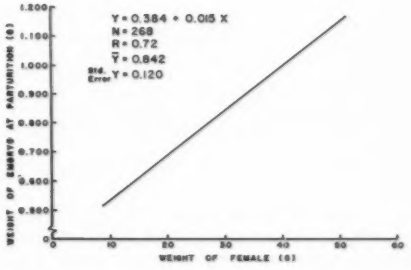

FIG. 4. Regression of the weight of *Cymatogaster aggregata* embryos at parturition compared to weight of 24 parent females.

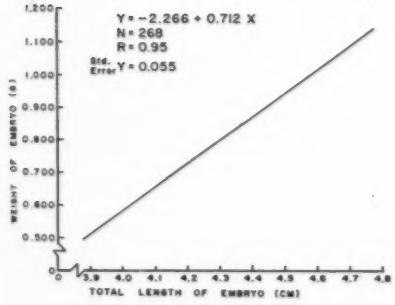

FIG. 5. Regression of the weight of *Cymatogaster aggregata* embryos compared to their total length at birth.

DISCUSSION

Our data show that shiner perch females born during the summer reach maturity and give birth to young during their second summer. This finding agrees with that of Suomela (MS, 1931) but not with that of Gordon (MS, 1965). The latter author reported that yearling females did not contain embryos, but more than 99% of his fish 2 years of age and older had embryos. Unfortunately, he did not state the type of length measurement he used; however,

we assume it was fork length because the relationship of female length to embryo number that he found was similar to the relationship that we obtained using fork length. If this assumption is correct, then our yearling fish are about 2 cm longer than Gordon's fish of the same age. It appears, therefore, that maturity of shiner perch may be dependent more on size than on age. The difference in size between our yearling fish and those of Gordon from British Columbia could be due to some environmental factor, perhaps temperature, that affects growth.

The relationship of female parent size to embryo size at parturition has not been studied previously in detail. Suomela (MS, 1931) reported that the standard length of embryos at birth was 2.7–3.8 cm, but he estimated these lengths from the lengths of unborn embryos. Our study complements that of Wiebe (1968b), who made a general statement that the largest females gave birth to the largest offspring and the greatest number of offspring. Neither Suomela nor Wiebe related embryo length at birth to female parent length.

We found that fecundity is directly related to female parent size. The same relationship was reported for shiner perch from California (Eigenmann, 1892), Washington (Suomela, MS, 1931), and British Columbia (Gordon, MS, 1965). However, our equations for fecundity are not necessarily applicable throughout the geographic range of the species.

ACKNOWLEDGMENTS

We wish to thank Mr Dennis E. Anderson and Mr Nelson E. Stewart, Oregon State University, for help in reading the fish scales, and Dr Roger G. Petersen, Oregon State University, for help with the statistical analyses.

This study was supported by Public Health Service research grant CC 00303, from the National Communicable Disease Center, Atlanta, Georgia, and constitutes part of thesis research conducted by the senior author at Oregon State University, Newport, Ore., USA.

REFERENCES

CARLISLE, J. G., JR., J. W. SCHOTT, AND N. J. ABRAMSON. 1960. The barred surfperch (*Amphistichus argenteus* Agassiz) in southern California. Calif. Dept. Fish Game, Fish Bull. 109. 79 p.

EIGENMANN, C. H. 1892. *Cymatogaster aggregatus* Gibbons; a contribution to the ontogeny of viviparous fishes. Bull. U.S. Fish Comm. 12: 401–478.

GORDON, C. D. MS, 1965. Aspects of the age and growth of *Cymatogaster aggregata* Gibbons. M.S. Thesis. Univ. British Columbia, Vancouver, B.C. 90 p.

HUBBS, C. L. 1921. The ecology and life-history of *Amphigonopterus aurora* and of other viviparous perches of California. Biol. Bull. 40: 181–209.

SUOMELA, A. J. MS, 1931. The age and growth of *Cymatogaster aggregatus* Gibbons collected in Puget Sound Washington. M.S. Thesis. Univ. Washington, Seattle, Wash. 43 p.

SWEDBERG, S. E. MS, 1965. Age-fecundity relationships in the striped seaperch *Embiotoca lateralis* from Yaquina Bay Oregon. M.S. Thesis. Oregon State Univ., Corvallis, Oregon. 41 p.

WARES, P. G. MS, 1968. Biology of the pile perch (*Rhacochilus vacca*). M.S. Thesis. Oregon State Univ., Corvallis, Oregon. 73 p.

WIEBE, J. P. 1968a. The effects of temperature and day length on the reproductive physiology of the viviparous seaperch, *Cymatogaster aggregata* Gibbons. Canadian J. Zool. 46: 1207–1219.

1968b. The reproductive cycle of the viviparous seaperch, *Cymatogaster aggregata*. Canadian J. Zool. 46: 1221–1234.

Caloric and Carbon Equivalents of Zooplankton Biomass[1]

TREVOR PLATT, VIVIEN M. BRAWN, AND BRIAN IRWIN

Fisheries Research Board of Canada
Marine Ecology Laboratory, Bedford Institute, Dartmouth, N.S.

PLATT, TREVOR, VIVIEN M. BRAWN, AND BRIAN IRWIN. 1969. Caloric and carbon equivalents of zooplankton biomass. J. Fish. Res. Bd. Canada 26: 2345–2349.

In a study throughout 1 year at a station in St. Margaret's Bay, N.S., the caloric content per unit dry weight of zooplankton showed a fivefold variation. Along with all the available information from the literature, the data indicated that the dependence of caloric content of zooplankton (y) on per cent organic matter (x) could be described by the equation $y = -3370 + 136\,x - 0.514\,x^2$. The caloric content may be predicted from per cent ash (A) and per cent carbon (C) by the equation $y = 1351 + 106\,C - 21.2\,A$. The relative stability of the caloric or carbon standing stock of zooplankton per unit water volume, compared with numbers or dry weight, may be an indication of some kind of regulatory mechanism controlling energy flow.

PLATT, TREVOR, VIVIEN M. BRAWN, AND BRIAN IRWIN. 1969. Caloric and carbon equivalents of zooplankton biomass. J. Fish. Res. Bd. Canada 26: 2345–2349.

La biomasse, la teneur en calories et la teneur en carbone du zooplancton ont été étudiées dans la Baie de St. Margaret, Nouvelle Écosse, à une station au cours de l'année 1967. La teneur en calories par unité de poids sec n'était pas constante pendant l'année; la valeur la plus élevée est cinq fois plus grande que la plus faible enregistrée. L'équation suivante exprime la relation existant entre la teneur en calories du zooplancton (y) et le per cent de matière organique (x) : $y = -3370 + 136\,x - 0.514\,x^2$. L'équation de regression de la teneur en calories (y), par rapport au per cent de carbone (C) et au per cent des cendres (A), est de la forme: $y = 1351 + 106\,C - 21.2\,A$. La stabilité relative des résultats obtenus avec le "standing stock" de calories ou de carbone par m³, comparée avec ceux obtenus pour le nombre d'individus ou le poids sec peut indiquer qu'il existe quelques sortes de mécanismes régulateurs controlant le flux d'énergie.

Received December 19, 1968

INTRODUCTION

IN STUDIES OF ENERGY FLOW in marine ecosystems it is often required to convert values of zooplankton biomass (expressed as dry weight) into more general units such as weight of organic carbon or quantity of calories. There is little available information on which to base such conversions (Curl, 1962; Beers, 1966; Comita et al., 1966; Ostapenya et al., 1967), and, to our knowledge, there is no previous study which considers all three quantitites (dry weight, carbon, and calories) for zooplankton on a seasonal basis.

[1]Bedford Institute Contribution 161 CCIBP 127.

Printed in Canada (J1317)

MATERIALS AND METHODS

The samples described constitute a 12-month series collected at a single station in St. Margaret's Bay, N.S. The station (64°02′W, 44°35′N) was 1 mile east of Mill Cove Wharf, at a depth of 58 m. Zooplankton samples were collected on 48 occasions during 1967, the interval between samples varying with the time of year.

Collections for chemical analysis were made by means of vertical tows using a metered net, 0.5 m in diameter and 366 μ in mesh size (#2). The design of the bucket allowed the catch to be trapped on a disc of 366 μ nylon mesh. The catch from each tow (approx 25 g wet weight) was washed for between 60 and 120 sec with distilled water, then transferred from the nylon disc to a plastic bag.

On each occasion five replicate tows were made from 50 m to the surface, and the catches accumulated in the same plastic bag, which was frozen at −20 C until required for analysis. The coefficient of variation of the catch of individual tows was estimated to be 10% on the basis of 10 additional replicate hauls. Assuming that the analytical error is small compared with the sampling error, this would imply that the 95% confidence limits on the observations are $\pm 2.75\ (0.1/\sqrt{5})\ \bar{x}$, or ±12% of the mean.

The frozen samples were freeze-dried and the total dry weight measured. About 5 mg of the freeze-dried material were used for carbon analysis by the wet-combustion method, essentially as described by Strickland and Parsons (1965). About 0.5 g were heated to 600 C for 30 min (or constant weight) for determination of ash content. Finally, about 1 g was used for measurement of caloric content using a Parr adiabatic bomb calorimeter (Brawn et al., 1968). Carbon and ash determinations were always made in triplicate, calorie determinations in duplicate.

In addition to the collections for chemical analysis, a further metered tow was made on each occasion and the catch preserved in 5% formalin. These samples were analysed for species composition by the Canadian Oceanographic Identification Centre, under Dr D. Faber.

Evaluation of Effect of Washing

Most authors who have studied the chemical composition of zooplankton have washed their samples with distilled water to remove inorganic salts that might interfere with subsequent analysis. This practice has been regarded as undesirable in that there is a risk of osmotic damage to delicate animals with consequent leaching of unknown amounts of organic matter, but acceptable provided it is done quickly enough. To evaluate the effect of distilled water washing we made the following simple test.

A sample of mixed plankton was collected by surface towing with a 150-μ (#10) mesh net in Bedford Basin. The catch was strained through #10 mesh and divided into six subsamples of approximately 50 g wet weight. Each subsample was placed into a 1-liter beaker, which was then filled with distilled water, and stirred. After 0.5 min the contents of one beaker were strained through #10 nylon mesh and the plankton transferred to a plastic bag. The other beakers were stirred occasionally and the plankton strained after 2, 5, 10, 20, and 60 min. The plastic bags were frozen immediately and stored at −20 C until the contents were freeze-dried and analysed for carbon and ash content as described above. There was no significant difference ($P = 0.05$) in the carbon results between samples subjected to different periods of washing from 30 sec to 60 min. The ash content showed no significant change between 2 min and 60 min, but was significantly lower after 2 min washing than after only 30 sec. This suggests that inorganic salts are not fully removed from bulk samples, of about 50-g wet weight, after 30 sec washing, and that washing should be extended to 2 min, which is possible without detriment to the carbon measurement. We expect that for smaller samples and individual zooplankters the salt could be fully removed in 30 sec.

RESULTS AND DISCUSSION

The detailed results, showing changes in species composition, numbers of individuals, dry weight, carbon content, and calorific value are tabulated in Platt and Irwin (MS, 1968). A high degree of correlation was found between

carbon content and caloric content ($r = 0.94$; 21 df); this implies that the following remarks about changes in energy content will be true, in general, for the carbon content.

There was a fivefold variation through the year in the energy equivalent of the zooplankton, expressed as cal/g dry wt. Most of this variation can be attributed to changing species composition (Curl, 1962; Beers, 1966) and to changing age distribution within species (Comita et al., 1966) throughout the year. It is evident that there is no single conversion factor that will serve to convert biomass of zooplankton, expressed as dry weight, to its energy equivalent. The time of year and the species composition should be taken into account.

Although there is no single satisfactory conversion factor between zooplankton biomass and caloric content, the *carbon* content of the zooplankton can evidently be used as a reliable predictor of the energy equivalent, for any season. The regression equation is:

$$\text{cal/g dry wt} = -227 + 152(\%\ \text{carbon}).$$

The multiple correlation coefficient among calories, carbon content, and ash content was 0.98 (16 df). If both the carbon and ash contents are known, the following equation may be used to predict caloric content:

$$\text{cal/g dry wt} = 1351 + 106(\%\ \text{carbon}) - 21.2(\%\ \text{ash}).$$

The standard error of the estimate is 253 cal.

Relationship Between Caloric Content and Organic Matter of Zooplankton

Ostapenya and Sergeev (1963) point out that caloric content per unit dry matter of zooplankton is controlled by two factors: variations in the ash content, and variations in the caloric content of the organic matter. The latter is determined largely by the fat content, so that when the proportion of fat is close to average (10–14%) the calorific value of zooplankton will be determined primarily by the ash content. Figure 1 shows all the available information on the dependence of zooplankton calories (direct measurement) on the organic content (100 — per cent ash in the dry matter). The observations of Ostapenya et al. (1967) were made in the Red Sea, Gulf of Aden, Gulf of Mexico, and equatorial waters of the Atlantic Ocean. The measurements were made on formalin-fixed samples (species composition not given). Pappenhoffer (1967) studied the caloric content of juveniles of *Artemia*. The remaining data in Fig. 1 are from St. Margaret's Bay.

All of the data shown in Fig. 1 were combined and fitted to two different equations. The best straight line fit has the equation $y = -1244 + 67.1\,x$ where y is cal/g dry wt and x is the per cent organic matter in the dry material. The scatter about the line is greatest at low organic content, and the data throughout the range can better be fitted to a second order equation of the

form $y = a + bx + cx^2$. The fitting was done by the method of Glass (1967). The best fit was found to be:

$$y = -3370 + 136\,x - 0.514\,x^2.$$

If a straight line be fitted to the St. Margaret's Bay data alone, the best fit has the equation:

$$y = -1190 + 58.5\,x.$$

The lower slope of this line, compared with that for *all* the available data, is due to the influence of Hydromedusae and ctenophores, which largely account for the lower values in the St. Margaret's Bay samples. These watery animals are not only low in organic matter content, but especially low in fats, which make up an important fraction of the caloric content of other animal groups.

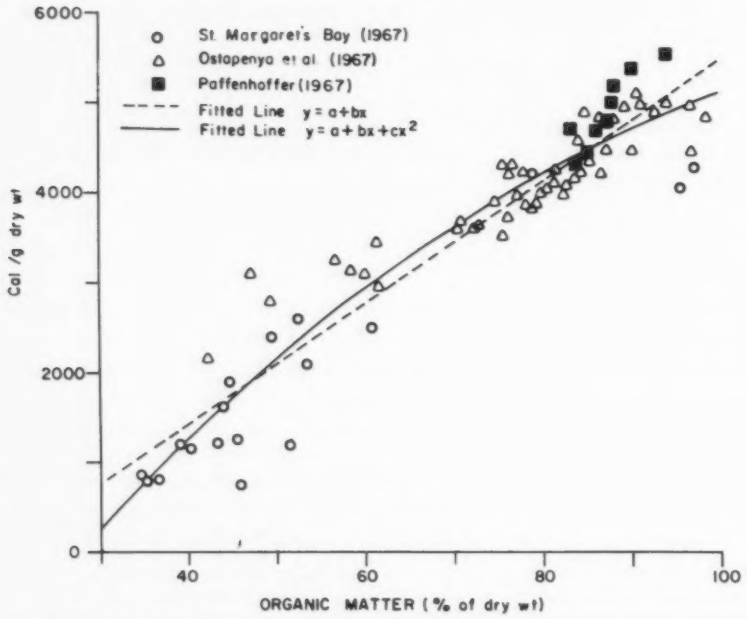

FIG. 1. Dependence of caloric content of zooplankton on the per cent organic matter, based on all available data.

From the point of view of studies of energy flow in biological communities it is of considerable interest that seasonal changes in total zooplankton calories per unit volume of water were appreciably lower than any other available index of zooplankton standing stock. Not unexpectedly, the standing stock of zooplankton, in terms of numbers of individuals taken, varied from 20 to 2800/m³, a 100-fold variation. The standing stock measured as dry weight,

however, showed only a 14-fold variation, suggesting that the size and numbers of species tend to change in some compensatory fashion. It now appears that the fivefold seasonal variation in caloric content of the dry weight is also an expression of some type of compensatory mechanism, since seasonal changes in the carbon or caloric content of zooplankton, per unit water volume, are only of the order of seven- to eightfold, half that shown by the dry weight index. Odum and Smalley (1959) found a similar phenomenon among populations of grasshoppers and snails and attributed it to some form of homeostatic mechanism controlling population energy flow. From our study we can see some evidence of a similar regulation of the energy flow in zooplankton; hence, although there are remarkable seasonal progressions in the character in which energy is presented to higher trophic levels, the amounts made available remain relatively constant.

ACKNOWLEDGMENTS

We are indebted to Dr D. Faber and the Canadian Oceanographic Identification Centre, Ottawa, for sorting the samples for species composition. It is a pleasure to thank Drs L. M. Dickie, E. Kott, I. A. McLaren, and K. H. Mann, who read and criticized drafts of the manuscript.

REFERENCES

BEERS, J. R. 1966. Studies in the chemical composition of the major zooplankton groups in the Sargasso Sea off Bermuda. Limnol. Oceanog. 11: 520–528.

BRAWN, V. M., D. L. PEER, AND R. J. BENTLEY. 1968. Caloric content of the standing crop of benthic and epibenthic invertebrates of St. Margaret's Bay, Nova Scotia. J. Fish. Res. Bd. Canada 25: 1803–1811.

COMITA, G. W., S. M. MARSHALL, AND A. P. ORR. 1966. On the biology of *Calanus finmarchicus*. XIII. Seasonal change in weight, calorific value and organic matter. J. Marine Biol. Assoc. U.K. 46: 1–17.

CURL, H. 1962. Analyses of carbon in marine plankton organisms. J. Marine Res. 20: 181–188.

GLASS, N. R. 1967. A technique for fitting non-linear models to biological data. Ecology 48: 1010–1013.

ODUM, E. P., AND A. E. SMALLEY. 1959. Comparison of population energy flow of a herbivorous and a deposit-feeding invertebrate in a salt marsh ecosystem. Proc. Natl. Acad. Sci. U.S. 45: 617–622.

OSTAPENYA, A. P., AND A. I. SERGEEV. 1963. [The caloric content of the dry substance of aquatic invertebrates used as food by fish.] Vopr. Ikhtiol. 3: 177–183. (FRB Transl. Ser. 874.)

OSTAPENYA, A. P., L. M. SUSHCHENYA, AND N. N. KHMELEVA. 1967. Caloricity of plankton from the tropical zone of the ocean. Okeanol. Keanologiza 6: 1100–1107. (In Russian, English abstr.)

PAPPENHOFFER, G. A. 1967. Caloric content of the larvae of the brine shrimp. Helgoländer Wiss. Meeresuntersuch. 16: 130–135.

PLATT, T., AND B. IRWIN. MS, 1968. Primary productivity measurements in St. Margaret's Bay. 1967. Fish. Res. Bd. Canada. Tech. Rept. 77.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1965. A manual of sea water analysis. 2nd ed. revised. Bull. Fish. Res. Bd. Canada 125. 203 p.

Serum Transferrin Systems and the Hemoglobins of the Pacific Halibut (*Hippoglossus stenolepis*)

H. Tsuyuki, E. Roberts, and E. A. Best[1]

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver 8, B.C.

Tsuyuki, H., E. Roberts, and E. A. Best. 1969. Serum transferrin systems and the hemoglobins of the Pacific halibut (*Hippoglossus stenolepis*). J. Fish. Res. Bd. Canada 26: 2351–2362.

Based on starch-gel electrophoretic analyses of serum proteins of 1092 specimens of Pacific halibut sampled from the eastern Bering Sea and northeastern Pacific Ocean southward to southern British Columbia, three molecular species of transferrins were encountered. A fourth rare type was postulated to explain the observation of some phenotypes involving this transferrin. These transferrins, either singly or in combinations of two, accounted for the theoretically possible 10 phenotypes of which 8 were actually observed. Hereditary control by four codominant alleles (Tf^A, Tf^B, Tf^C, and Tf^D) is postulated to explain the heterogeneity of the transferrin patterns. The collections were arbitrarily divided into 10 geographic areas and gene frequency analyses were used to determine population structure. Phenotypic distribution was shown to be independent of age and sex. Of the 10 areas, only the collection from southeastern Alaska proved not to be homogeneous. Preliminary analysis of blood hemoglobins indicated that these proteins are not of value in population analyses.

Received March 31, 1969

INTRODUCTION

Polymorphisms in the iron-binding serum transferrins have been investigated in a number of mammals, reptiles, and birds. In fish, similar polymorphisms have been observed in gadoids (Møller and Naevdal, 1966a), scombroids (Barrett and Tsuyuki, 1967; Fujino and Kang, 1967), eels (*Anguilla anguilla*) (Fine et al., 1964; Drilhon et al., 1966), carp (*Cyprinus carpio*) (Creyssel et al., 1964), bigmouth buffalofish (*Ictiobus cyprinellus*) (Koehn and Johnson, 1967), sprat (*Sprattus sprattus*) (Naevdal, 1968), plaice (*Pleuronectes platessa*) (De Ligny, 1966), cod (*Gadus morhua*) (Møller, 1966, 1968), and 12 other fishes (Møller and Naevdal, 1966b). Of these, genetic control of transferrin polymorphism has been demonstrated in cod, scombroids, plaice, sprat, gadoids, bigmouth buffalofish, and eels. The objective of most of these transferrin polymorphism studies has been to elucidate the structure of fish populations. The results, however, have not generally attained the high expectations hoped for as the frequencies of genes responsible for the formation of specific molecular species of serum transferrins have not displayed the large geographic variations

[1]Present address (E.A.B.): International Pacific Halibut Commission, Seattle, Washington, USA.

Printed in Canada (J1429)

observed, for example, in the hemoglobins (HbI′) of the Atlantic cod (Sick, 1965; Frydenberg et al., 1965). Although genetically linked polymorphisms in hemoglobins have been observed in *Sebastodes crameri* (Tsuyuki et al., 1968), sprat (Naevdal, 1968), and in Atlantic cod (Sick, 1965; Frydenberg et al., 1965), the last two species represent the only reported studies in which statistically significant numbers were analyzed for the purpose of population analysis. The popular choice of serum transferrins for gene frequency studies resides in part in sampling convenience. Frozen storage does not usually affect the electrophoretic mobility characteristics of serum transferrins whereas blood hemoglobins of fishes are difficult to store and electrophoretic analyses must be carried out either immediately or within a few days at the most.

This report describes the serum transferrin systems of the Pacific halibut (*Hippoglossus stenolepis*) in the northeastern Pacific Ocean and the Bering Sea as well as the blood hemoglobin electropherograms of a few specimens from Hecate Strait.

MATERIALS AND METHODS

Collection of Fish

The 1092 blood samples analyzed were collected during the summer months of 1967 and 1968 from 10 selected areas, ranging from the Bering Sea to the southwestern coast of British Columbia (Fig. 1, Table 1). All fish were captured by means of otter trawl nets by the vessels *Don Edwards*, *Harmony*, *Karen T*, and *St. Michael*, all under charter to the International Pacific Halibut Commission, and by the *Great Northern III* and the *G. B. Reed* through the Fisheries Research Board of Canada. Where possible, blood samples were collected from small fish with a view towards minimizing any biases due to the increased migratory tendency expected of larger fish. The minimum size of fish sampled was governed by the quantity of blood required for electrophoretic analysis. Each collection was made from a rather small geographic location and restricted to a period of 2 consecutive days to minimize recruitment from adjacent populations.

Collection of Blood

Shortly after fish were brought aboard the vessel, whole blood was withdrawn from the caudal artery into a nonheparinized Becton and Dickinson 4-ml-capacity Vacutainer tube (No. 3274) equipped with a 1-inch, 22-gauge, holder-needle assembly. After sufficient time was allowed for clotting, the blood was centrifuged in a Phillips-Drucker centrifuge directly in the Vacutainer tubes, and the clear serum was transferred with disposable pipettes to 2-ml disposable beakers (No. B2714)[1] fitted with polyethylene caps (No. B2714) and frozen for subsequent analysis at this laboratory.

Blood hemoglobins were obtained by a previously described method (Tsuyuki et al., 1968) and separated electrophoretically immediately.

Electrophoresis

The serum proteins were separated by electrophoresis on starch-gel, consisting of a 13% starch[2] solution in .005 M tris (hydroxymethyl) amino methane, *p*H 8.0, for 110 min at 180 v in an apparatus previously described by Tsuyuki et al. (1966). Bridge buffer solution consisted of .05 M tris (hydroxymethyl) amino methane of the same *p*H.

---

[1]Obtained from Scientific Products, Evanston, Illinois.

[2]Obtained from Connaught Medical Research Laboratory, University of Toronto, Toronto, Ont.

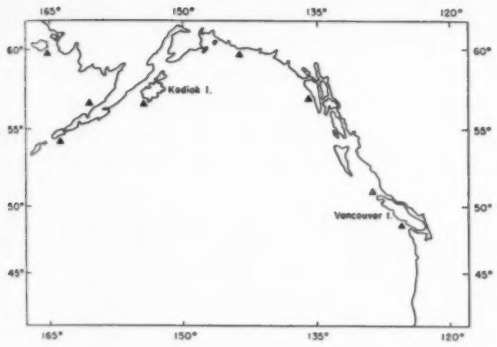

FIG. 1. Eastern Bering Sea and eastern Pacific Ocean, from which halibut samples were collected during 1967–68.

TABLE 1. Location of geographic areas of sample collections.

Area	Lat	Long
NE Bering Sea	59:30	165:30
SE Bering Sea	57:00	161:00
Unimak Is.	54:38	163:30
Alitak Bay	56:48	154:18
Kayak Is.	59:54	144:33
Shelikof Bay	57:08	135:49
Cape Scott	51:09	128:46
La Perouse Bank	48:35	125:37
Queen Charlotte Strait	51:00	128:20
Skidegate	53:25	131:00

Blood hemoglobins were separated by starch-gel electrophoresis for 2 hr at 160 v in 0.023 M sodium borate buffer, *p*H 8.5, containing 0.025% disodium ethylenediaminetetraacetic acid and using bridge buffer solution of 0.3 M sodium borate at the same *p*H (Tsuyuki et al., 1966).

Specific serum transferrins were located by autoradiography (Giblett et al., 1959). Three volumes of serum were mixed with one volume of iron-59 sulfate[4] (approximately 30 μc/ml), and equilibrated for about 30 min, and the proteins were separated by starch-gel electrophoresis. At the end of separations, the gel slices were transferred to a specially designed lightproof holder, somewhat similar in construction to a contact print holder, overlaid with a thin sheet of mylar, followed by a G.E. no-screen medical X-ray film. The entire unit was then placed in a freezer and, depending on the state of decay of iron-59 sulfate, exposed for 3–14 days before processing.

RESULTS AND DISCUSSION

With few exceptions, blood hemoglobins of the Pacific halibut sampled at the Horseshoe and Bonilla grounds in Hecate Strait exist as a two-component system, one migrating anodically and the other cathodically at *p*H 8.5 (Fig. 2). Three out of 66 specimens displayed two additional components for a total of four. The observations of polymorphic patterns were not sufficient

[4]Obtained from Abbott Laboratories, North Chicago, Illinois 60064.

either in numbers or in different geographic areas to establish genetic relationships. Indications are, however, that the hemoglobins of this species are essentially homogeneous and unlikely to be of value in population studies.

The most commonly encountered serum transferrins, visualized autoradiographically, appeared as three electrophoretically distinct zones, designated in this investigation as A, B, and C in the order of increasing anodical mobility. In all specimens, the zones appeared either singly or in combinations of two, resulting in 6 phenotypes, AA, BB, CC, AB, AC, and BC, the theoretically possible combinations (Fig. 3). To account for the observation of two more rare phenotypes, transferrin zone D, though not actually observed in the homozygous state, was hypothesized, giving additional phenotypes AD, BD, DD, and CD, of which the last two were not observed in this investigation. The gene responsible for the formation of transferrin zone D is rare, at least in the geographic areas investigated.

The distributions of the various transferrin patterns in 1092 specimens collected from 10 different geographic areas are presented in Table 2. Samples from Alitak, Kayak, and Shelikof represent more than one collection from the same area. The expected numbers of phenotypes involving only transferrins A, B, and C are calculated according to the Hardy–Weinberg law. Only the observed distributions of phenotypes involving transferrin D are presented. Chi-square tests show that only the distributions from Shelikof Bay 1967 and from La Perouse grounds 1968 deviate significantly from those expected in randomly breeding homogeneous populations, although those from southeast Bering Sea and Alitak 1967 approach the 5% level of significance. In the 1967 collection from Shelikof, the observation of a single homozygote AA accounts for most (8.915) of the high chi-square value (12.719). If the contribution of the homozygote AA is omitted, a chi-square value of 3.805 is obtained and is well within the 5% level of significance. When the distribution of the six phenotypes in the pooled sample of 1092 is considered, a chi-square value of 6.926 is obtained, indicating a significant departure from the Hardy-Weinberg distribution. The scarcity of phenotype AC ($\chi^2 = 4.161$) and to some extent an excess of phenotype AB (1.895) account for most of the high chi-square value obtained for the pooled sample. If the chi-square is recalculated by omitting the 100 specimens from La Perouse grounds, a nonsignificant value of 3.095 (2 df) is obtained, indicating that the sample from this area is responsible for the observed departure from the Hardy–Weinberg distribution.

In Table 3, the data from Table 2 are presented as gene proportions. Collections from Queen Charlotte Strait 1968 and Shelikof (August 1968) deviate significantly from the expected values calculated from the pooled samples whereas those from southeast Bering Sea and Shelikof (July 1968) approach the 5% level of significance. The sum of chi-square is 33.588 (26 df, $P = .25–.10$), a value slightly above the 5% level of significance. When the three collections from Shelikof are combined, the chi-square value of

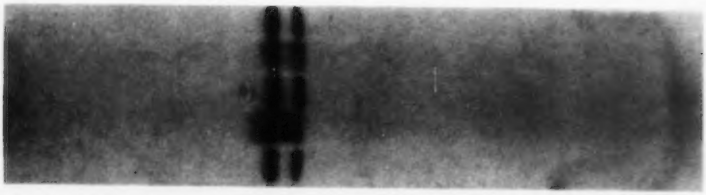

FIG. 2. Starch-gel zone electropherogram of halibut hemoglobins. In this and Fig. 3, the cathode is to the left and the anode to the right.

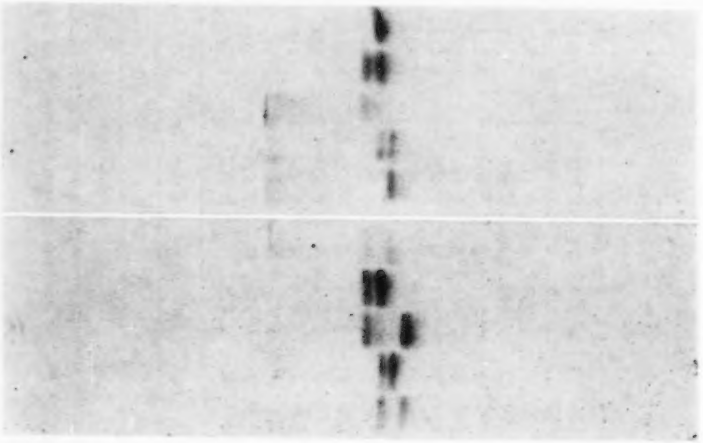

FIG. 3. Autoradiographs of serum transferrin patterns of halibut obtained by starch-gel zone electrophoresis. Transferrin zones are labelled A, B, C, and D in the order of increasing anodic mobility. The phenotypic expresssion of the eight observed combinations of the 10 possible types are shown in the right column. Phenotypes DD and CD were not observed.

TABLE 2. Phenotypic composition of Pacific halibut serum transferrins as compared with the Hardy–Weinberg proportions.

Collection	No. of samples	Phenotypes: AA Obs.[a]	AA Exp.[a]	BB Obs.	BB Exp.	CC Obs.	CC Exp.	AB Obs.	AB Exp.	AC Obs.	AC Exp.	BC Obs.	BC Exp.	Rare types: DD	AD	BD	CD	χ^2[b]	Probability of worse fit (2 df)
NE Bering Sea 1968	90	0	.069	46	46.944	5	5.625	4	3.601	1	1.247	34	32.500	0	0	0	0	.320	.900–.750
SE Bering Sea 1968	98	0	.010	50	53.626	3	6.125	2	1.479	0	.500	43	36.245	0	0	0	0	3.792	.250–.100
Unimak Is. 1968	98	0	.094	50	50.705	5	6.125	4	4.314	2	1.499	37	35.246	0	0	0	0	.588	.750–.500
Alitak Bay 1967	60	0	.038	25	28.698	2	4.818	3	2.075	0	.850	30	25.512	0	0	0	0	4.215	.250–.100
Alitak Bay 1968	99	0	.091	50	48.782	8	7.088	4	4.212	2	1.605	35	37.195	0	0	0	0	.476	.900–.750
Kayak Is. 1967	59	0	.038	31	30.609	4	3.811	2	2.159	1	.762	21	21.606	0	1	0	0	.155	.950–.900
Kayak Is. 1968	100	0	.090	54	53.290	7	5.760	5	4.380	1	1.440	33	35.040	0	0	0	0	.707	.750–.500
Shelikof Bay 1967	66	1	.0924	29	26.723	10	7.003	3	3.175	0	1.625	23	27.356	0	2	0	0	12.719	< .01
" " July 1968	100	0	.040	41	42.250	11	10.890	4	2.600	0	1.320	44	42.900	0	0	0	0	2.263	.500–.250
" " Aug. 1968	58	0	.429	25	24.244	6	4.141	8	6.465	2	2.672	17	20.038	0	0	1	0	2.281	.500–.250
Skidegate 1968	47	0	.193	22	22.466	3	2.811	5	4.147	1	1.467	16	15.897	0	0	1	0	.540	.900–.750
Queen Charlotte Strait 1968	93	0	.270	47	48.267	5	4.743	9	7.196	1	2.255	31	30.256	0	0	0	0	1.486	.500–.250
Cape Scott 1968	24	0	.010	10	10.666	2	2.345	1	.666	0	.312	11	9.999	0	0	0	0	.682	.750–.500
La Perouse 1968	100	0	.203	42	47.610	4	7.020	9	6.210	0	2.385	45	36.570	0	0	0	0	7.745	.025–.010
Pooled	1092	1	1.310	522	533.660	75	77.314	63	52.981	11	20.159	420	406.138					6.926	.050–.025
Pooled less La Perouse	992	1	1.091	480	486.179	71	70.234	54	46.822	11	17.856	375	369.619					3.905	.250–.100

[a]Obs. = observed; exp. = expected, in all tables.

[b]$\chi^2_{\text{heterogeneity}} = \chi^2_{\text{sum}} - \chi^2_{\text{total}} = 37.967 - 6.926 = 31.041$ ($P = .250 - .100$, 26 df).

TABLE 3. Genic composition of Pacific halibut transferrins.

Collection	Total no. genes	Tf^A Obs.	Tf^A Exp.	Tf^B Obs.	Tf^B Exp.	Tf^C Obs.	Tf^C Exp.	χ^2
NE Bering Sea 1968	180	5	6.264	130	125.852	45	47.885	.565
SE Bering Sea 1968	196	2	6.821	145	137.038	49	52.141	4.059
Unimak Is. 1968	196	6	6.821	141	137.038	49	52.141	.402
Alitak Bay 1967	120	3	4.176	83	83.901	34	31.923	.476
Alitak Bay 1968	198	6	6.890	139	138.437	53	52.673	.119
Kayak Is. 1967	118	3	4.106	85	82.503	30	31.391	.435
Kayak Is. 1968	200	6	6.960	146	139.835	48	53.205	.913
Shelikof Bay 1967	132	5	4.593	84	92.291	43	35.115	2.551
" " July 1968	200	4	6.960	130	139.835	66	53.205	5.027
" " Aug. 1968	116	10	4.037	75	81.104	31	30.859	9.270
Skidegate 1968	94	6	3.271	65	65.723	23	25.006	2.446
Queen Charlotte Strait 1968	186	10	6.473	134	130.047	42	49.481	5.971
Cape Scott 1968	48	1	1.670	32	33.560	15	12.769	.731
La Perouse Bank 1968	200	9	6.960	138	139.835	53	53.205	.623
Pooled	2184	76		1527		581		33.588[a]
Alitak Bay 1967–68	318	9	11.066	222	222.338	87	84.596	.454
Kayak Is. 1967–68	318	9	11.066	231	222.338	78	84.596	1.237
Shelikof Bay 1967–68	448	19	15.590	289	313.231	140	119.179	6.258
Pooled 2-year samples								22.747[b]

[a] $\chi^2_{sum} = 33.588$ (26 df, $P = .25–.10$)
[b] $\chi^2_{sum} = 22.747$ (18 df, $P = .25–.10$)

6.258 (2 df) still indicates a significant deviation from the overall gene frequencies (Table 3).

For the analysis for distribution of phenotypes by age (Table 4), the 2- to 5-year-old groups were selected from the total samples as no single year-class was collected in sufficient numbers from the various geographic areas, with the exception of one, for meaningful analysis by locations. Age-groups under 2 and over 5 were sampled infrequently. In the four year-classes, the observed values of the six phenotypes do not deviate significantly from the expected values, calculated from the gene frequencies of individual year groups, in the entire range studied. The Shelikof Bay collections of July 1968 in which the 4-year fishes predominated, followed by a high proportion of 2-year fishes in the August collection, made possible a comparison of phenotypic distributions in two different age-groups from a single area with those of the whole range examined. Nonsignificant chi-square values of 2.784 ($P = .250–.100$, 2 df) for the 2-year-olds and 0.457 ($P = .900–.750$, 2 df) for the 4-year-olds suggest that at least in this one area and for these 2 year-classes, the pattern of phenotypic distribution with age is comparable with that of the total sample. Although the heterogeneity chi-square (Table 4) is not significant, the ratio of observed to expected in phenotype AB suggests a general increase with increasing age

TABLE 4. Distribution of phenotypes by age.

Age (*years*)	Total obs.	Phenotypes												Gene frequency			χ^2,[a] (2 df)	Obs./exp. ratios of phenotypes				
		AA		BB		CC		AB		AC		BC										
		Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	q^A	q^B	q^C		BB	CC	AB	AC	BC
2	192	0	.292	91	90.048	16	14.630	11	10.253	4	4.147	70	72.576	.0390	.6848	.2760	.580	1.011	1.094	1.073	.965	.965
3	426	0	.233	219	221.946	27	27.605	17	14.399	3	5.112	160	156.598	.0234	.7218	.2546	1.705	.987	.978	1.181	.587	1.022
4	142	0	.176	64	66.243	12	11.261	10	6.816	0	2.812	56	54.613	.0352	.6830	.2816	4.634	.966	1.066	1.467	–	1.025
5	47	0	.211	20	20.440	5	4.785	2	1.316	0	.639	20	19.778	.0212	.6595	.3191	1.227	.978	1.045	1.520	–	1.011
Sum																	8.147					
Total	807	0	.646	394	398.335	60	58.023	40	32.926	7	12.589	306	304.078	.0291	.7026	.2682	4.774	.989	1.034	1.215	.556	1.006

[a] $\chi^2_{\text{heterogeneity}} = \chi^2_{\text{sum}} - \chi^2_{\text{total}} = 3.373$ ($P = .900 - .750$, 7 df).

reminiscent of the type of variation observed in the serum transferrin phenotypes of skipjack tuna from Hawaii (Fujino and Kang, 1967).

The chi-square test for independence of phenotypes and age (Table 5) indicates that the incidence of observed phenotypes BB, CC, AB, AC, and BC in each age-group are in good agreement with the proportions calculated

TABLE 5. Chi-square for independence of phenotypes and age.

Phenotypes		Age (*years*) 2	3	4	5	Total	χ^2	Probability (3 df)
BB	Obs.	91	219	64	20	394	1.451	.750–.500
	Exp.	93.740	207.985	69.328	22.947			
CC	Obs.	16	27	12	5	60	1.142	.900–.750
	Exp.	14.275	31.673	10.558	3.494			
AB	Obs.	11	17	10	2	40	2.326	.750–.500
	Exp.	9.517	21.115	7.038	2.330			
AC	Obs.	4	3	0	0	7	5.043	.250–.100
	Exp.	1.665	3.695	1.232	.408			
BC	Obs.	70	160	56	20	306	.475	.950–.900
	Exp.	72.803	161.532	53.844	17.822			
Total		192	426	142	47	807	10.437[a]	.900–.750

[a]15 df.

from the total sample. Both the individual chi-squares and their sums fall well above the 5% level of significance. Homozygote AA, encountered only once in 1092 samples, has been omitted in this analysis.

All samples in which sex was determined were pooled and the distribution of phenotypes by sex was determined (Table 7). The chi-square values of

TABLE 6. Chi-square for independence of phenotypes and sex.

Phenotype		Sex Male	Female	Total	χ^2	Probabilities (1 df)
AA	Obs.	0	1	1	.837	.500–.250
	Exp.	.455	.544			
BB	Obs.	181	217	398	.000	
	Exp.	181.391	216.608			
CC	Obs.	34	28	62	2.144	.250–.100
	Exp.	28.256	33.743			
AB	Obs.	19	22	41	.009	.900–.750
	Exp.	18.686	22.313			
AC	Obs.	3	6	9	.542	.500–.250
	Exp.	4.101	4.898			
BC	Obs.	139	175	314	.215	.750–.500
	Exp.	143.107	170.892			
Total		376	449	825	3.747[a]	.750–.500

[a]6 df.

TABLE 7. Distribution of phenotypes by sex.

Sex		Phenotypes AA	BB	CC	AB	AC	BC	Total obs.	Gene frequency q^A	q^B	q^C	χ^2	Probability of worse fit (2 df)
Male	Obs.		181	34	19	3	139	376	.0292	.6914	.2792	3.9322	.250–.100
	Exp.	.338	179.728	29.328	15.190	6.166	145.136						
Female	Obs.	1	217	28	22	6	175	449	.0334	.7026	.2639	1.6216	.500–.250
	Exp.	.494	221.626	31.250	21.103	7.902	166.489						

TABLE 8. Tf^A, Tf^B, and Tf^C gene frequencies in Pacific halibut.

	No. of genes in sample	Tf^A Observed frequency	Tf^A 95% confidence limits	Tf^B Observed frequency	Tf^B 95% confidence limits	Tf^C Observed frequency	Tf^C 95% confidence limits
NE Bering Sea 1968	180	.0277	.0038–.0516	.7222	.6568–.7876	.2500	.1867–.3133
SE Bering Sea 1968	196	.0102	0 –.0243	.7397	.6783–.8011	.2500	.1894–.3106
Unimak Is. 1968	196	.0306	.0065–.0547	.7193	.6564–.7822	.2500	.1894–.3106
Alitak Bay 1967	120	.0250	0 –.0530	.6916	.6089–.7743	.2833	.2027–.3639
Alitak Bay 1968	198	.0303	.0052–.0554	.7020	.6382–.7658	.2676	.2059–.3293
Kayak Is. 1967	118	.0254	0 –.0538	.7203	.6393–.8023	.2542	.1756–.3328
Kayak Is. 1968	200	.0300	.0063–.0537	.7300	.6685–.7915	.2400	.1808–.2992
Shelikof Bay 1967	132	.0378	.0053–.0703	.6363	.5542–.7184	.3257	.2457–.4057
" " July 1968	200	.0200	.0006–.0394	.6500	.5839–.7161	.3300	.2647–.3953
" " Aug. 1968	116	.0862	.0350–.1374	.6465	.5595–.7335	.2672	.1866–.3478
Skidegate 1968	94	.0638	.0144–.1132	.6914	.5980–.7848	.2446	.1578–.3314
Queen Charlotte Strait 1968	186	.0537	.0214–.0860	.7204	.6559–.7849	.2258	.1656–.2860
Cape Scott 1968	48	.0208	0 –.0612	.6666	.5333–.7999	.3125	.1814–.4436
La Perouse Bank 1968	200	.0450	.0162–.0738	.6900	.6259–.7541	.2650	.2038–.3262
All samples less Shelikof	1736	.0328		.7131	.6918–.7344	.2540	
Pooled Shelikof	448	.0424		.6450	.6007–.6893	.3125	

3.932 (P = .250–.100, 2 df) and 1.622 (P = .500–.250, 2 df) for male and female, respectively, indicate good agreement between the observed and expected numbers in both sexes. The number of samples in most collections was insufficient for a meaningful independent analysis by geographic areas. Individual chi-squares as well as their totals (Table 6) fall well above the 5% level of significance, indicating good agreement between the observed incidence of phenotypes in each sex and the expected values calculated from the proportions observed in the combined sexes.

The frequencies of the three alleles responsible for the three main transferrins, Tf^A, Tf^B, and Tf^C, are shown in Table 8. The frequencies of the minor transferrin, Tf^A, from the 14 collections vary from a low of .0102 in southeast Bering Sea to a high of .0862 in the Shelikof Bay collection of August 1968. The most abundant transferrin, Tf^B, ranged from a low of .6363 in the Shelikof Bay collection of 1967 to a high of .7397 in southeast Bering Sea. Low frequencies ranging from .6363 to .6500 were obtained in all three collections from the Shelikof Bay area, although the 95% confidence limits show overlap from collection to collection. The frequency of the most abundant Tf^B for the combined samples less Shelikof was calculated as 0.7131 with 95% confidence limits of 0.6918–0.7344; that for the pooled Shelikof Bay samples was calculated as 0.6450 with limits of 0.6007–0.6893, showing the absence of overlap in confidence intervals. Considered in conjunction with the chi-square value of 6.258 (P = .05–.02, 2 df) for independence from collection area (Table 3), the population of the under 6-year-old Pacific halibut in the Shelikof Bay area are markedly different from the remainder in this study. With the exception of this one area, no strikingly different cline in Tf^B frequencies was observed.

ACKNOWLEDGMENTS

We wish to express our appreciation to the following members of the International Pacific Halibut Commission: Messrs Paul H. Breen, Ian R. McGregor, and Daniel A. H. Unruh for collecting and processing blood samples at sea; and Mr F. Heward Bell, Director of Investigations, and Mr Richard J. Myhres for valuable advice on the halibut fishery and biology and for making this study possible. We are also indebted to Dr D. E. Kramer of the Fisheries Research Board of Canada for collecting samples aboard the *Great Northern III* and to Dr Conrad Wehrhahn, Institute of Fisheries, University of British Columbia, for his advice on statistical analyses.

REFERENCES

BARRETT, I., AND H. TSUYUKI. 1967. Serum transferrin polymorphism in some scombroid fishes. Copeia 1967: 551–557.

CREYSSEL, R., P. SILBERZAHN, G. RICHARD, AND Y. MANUEL. 1964. Étude du sérum de carpe (*Cyprinus carpio*) par électrophorèse en gel d'amidon. Bull. Soc. Chim. Biol. 46: 149–159.

DE LIGNY, WILHELMINA. 1966. Polymorphism of serum transferrin in plaice. X^e Congrès européen sur les groupes sanguins et le polymorphisme biochimique des animaux, Paris, 5–8 juillet. p. 373–378.

DRILHON, A., J. M. FINE, G. BOFFA, P. AMOUCH, AND J. DROUHET. 1966. Les groupes de transferrine chez l'anguille. Différences phénotypiques entre les anguilles de l'Atlantique et les anguilles Méditerranéennes. Compt. Rend. 262: 1315–1318.

FINE, J. M., A. DRILHON, P. AMOUCH, AND G. BOFFA. 1964. Existence de groupes sériques chez *Anguilla anguilla* L. Mise en évidence par électrophorèse et autoradiographie de plusieurs types de transferrines. Compt. Rend. 258: 753–756.

FRYDENBERG, O., DAG MØLLER, GUNNAR NAEVDAL, AND KNUD SICK. 1965. Hemoglobin polymorphism in Norwegian cod populations. Hereditas 53: 257–271.

FUJINO, K., AND T. KANG. 1967. Transferrin groups of tunas. Genetics 59: 79–91.

GIBLETT, E. R., C. G. HICKMAN, AND O. SMITHIES. 1959. Serum transferrins. Nature 183: 1589–1590.

KOEHN, R. K., AND D. W. JOHNSON. 1967. Serum transferrin and serum esterase polymorphisms in an introduced population of the bigmouth buffalofish (*Ictiobus cyprinellus*). Copeia 1967: 805–808.

MØLLER, D. 1966. Polymorphism of serum transferrin in cod. Fiskeridirektorat. Skrifter Ser. Havundersokelser 14: 51–60.

1968. Genetic diversity in spawning cod along the Norwegian coast. Hereditas 60: 1–32.

MØLLER, D., AND G. NAEVDAL. 1966a. Serum transferrins of some gadoid fishes. Nature 210: 317–318.

1966b. Transferrin polymorphism in fishes. X° Congrès européen sur les groupes sanguins et le polymorphisme biochimique des animaux, Paris, 5–8 juillet. p. 367–372.

NAEVDAL, G. 1968. Studies on hemoglobins and serum proteins in sprat from Norwegian waters. Fiskeridirektorat. Skrifter Ser. Havundersokelser 14: 160–182.

SICK, K. 1965. Hemoglobin polymorphism of cod in the Baltic and Danish Belt Sea. Hereditas 54: 19–48.

TSUYUKI, H., E. ROBERTS, R. H. KERR, AND A. P. RONALD. 1966. Micro starch gel electrophoresis. J. Fish. Res. Bd. Canada 23: 929–933.

TSUYUKI, H., E. ROBERTS, R. H. LOWES, W. HADAWAY, AND S. J. WESTRHEIM. 1968. Contributions of protein electrophoresis to rockfish (*Scorpaenidae*) systematics. J. Fish. Res. Bd. Canada 25: 2477–2501.

Growth Rate and Body Composition of Fingerling Sockeye Salmon, *Oncorhynchus nerka*, in relation to Temperature and Ration Size

J. R. BRETT, J. E. SHELBOURN, AND C. T. SHOOP

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

BRETT, J. R., J. E. SHELBOURN, AND C. T. SHOOP. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J. Fish. Res. Bd. Canada 26: 2363–2394.

The growth of young sockeye salmon (*Oncorhynchus nerka*) was studied at temperatures ranging from 1 to 24 C in relation to rations of 0, 1.5, 3, 4.5, and 6% of dry body weight per day, and at an "excess" ration. Optimum growth occurred at approximately 15 C for the two highest rations, shifting progressively to a lower temperature at each lower ration. The maximum growth rate for sockeye 5–7 months old was 2.6%/day; that for fish 7–12 months old was 1.6%/day. At 1 C a ration of 1.5%/day was sufficient to provide for a maximum growth rate of 0.23%/day. The maintenance ration was found to increase rapidly above 12 C, amounting to 2.6%/day at 20 C. No growth took place at approximately 23 C despite the presence of excess food.

Isopleths for gross and net food-conversion efficiencies were calculated. A maximum gross efficiency of 25% occurred in a small area with a center at 11.5 C and a ration of 4.0%/day; a maximum net efficiency of 40% occurred within a range of 8–10 C for rations of 1.5%/day down to 0.8%/day, the maintenance level.

Gross body constituents changed in response to the imposed conditions, varying in extreme from 86.9% water, 9.4% protein, and 1.0% fat for starved fish at 20 C to 71.3% water, 19.7% protein, and 7.6% fat on an excess ration at 15 C.

It is concluded on the basis of growth and food-conversion efficiency that temperatures from 5 to 17 C are most favorable for young sockeye, and that a general physiological optimum occurs in the vicinity of 15 C.

Received March 13, 1969

INTRODUCTION

THIS PAPER is the first of a series dealing with laboratory studies on the relation of environmental factors (abiotic and biotic) to the growth rate of young sockeye salmon, *Oncorhynchus nerka*. The aim is to obtain a broad understanding of the bioenergetics of this species throughout its life history. As such, the present work constitutes an extension of the research on metabolic rate and performance of sockeye salmon for which a considerable background of information concerning the effects of temperature, size, and swimming speed is available (Brett, 1964, 1965, 1967).

By relating food consumption, growth rate, and metabolic rate the energy budget can be determined, providing a measure of the efficiency of food conversion and of energy loss through metabolism and excretion (see Winberg,

Printed in Canada (J1409)

1956; Warren and Davis, 1967). Studies of this sort can be expected to contribute to an improved understanding of the food requirements of natural populations as well as provide fundamental information for fish-cultural practices. Many of the methods and approaches to animal energetics, conducted for years on warm-blooded vertebrates (e.g., Brody, 1945; Kleiber, 1961; Blaxter, 1965), may be applied with profit to studies on fish energetics.

The present experiments deal with the combined effects of temperature and ration on growth rate. Temperature has been shown repeatedly to be one of the most influential environmental factors affecting the growth of fishes (Baldwin, 1957; Brown, 1957; Donaldson and Foster, 1940; Haskell et al., 1956; Paloheimo and Dickie, 1966a, b; Strawn, 1961; Swift, 1964; West, 1966). When food is present in abundance an optimum temperature for growth has been recorded, varying among species and in the case of the desert pupfish (*Cyprinodon macularius*) significantly influenced by salinity (Kinne, 1960). Quantity and quality of food under ambient temperature conditions have been studied intensively, especially for artificial culturing of fish (e.g., Brown, 1957; Halver and Shanks, 1960; Hatanaka and Takahashi, 1960; Phillips et al., 1966). Davis and Warren (1968) reported that young chinook salmon (*Oncorhynchus tshawytscha*) weighing 0.6 g would consume a ration as high as 20% of their dry body weight per day. However, the *interacting* effect of ration and temperature on the growth rate of fish does not appear to have been the object of critical study except where either maintenance or ad libitum rations were involved (Brown, 1946; Pentelow, 1939).

It was hypothesized that the optimum temperature for growth would drop as the ration decreased, accompanied by a reduction in conversion efficiency. This was based on the supposition that the decrease in maintenance metabolism that accompanies reduced temperature would permit comparatively better growth at lower temperatures when the source of energy was restricted. The assumption would apply only for a poikilotherm if the temperature-dependent activity of digestive enzymes and growth processes did not exert such a controlling influence that the potential shift was inhibited. The presence of sharp peaks in the activity of digestive enzymes in relation to temperature has been demonstrated for the brown bullhead (*Ictalurus nebulosus*) by Smit (1967). Hoar (1966) further confirms this as a general phenomenon among poikilotherms (see also Jennings, 1965).

MATERIALS AND METHODS

The program of study was conducted over a period of 3½ years during which time some change in methods was adopted. The initial phase, dealing with the effect of temperature (5, 10, 15, 20, and 24 C) using excessive ration, lasted for 7 months (June 2, 1964–January 12, 1965). This series served to establish the pattern of the growth curve, to provide an estimate of the maximum growth rate at each temperature, and to assess the methods employed. Subsequent experiments involving reduced rations were performed from November 1, 1965, to February 3, 1966, at temperatures of 5, 10, and 15 C, and from December 11, 1966, to March 5, 1967, at temperatures of 1 and 20 C. The methodology for the last two experiments was essentially similar. A distinction will be made only between the two series — *A group*, on excess ration, and *B group*, including restricted rations — whenever this is pertinent to the presentation.

Source and Culturing of Fish

All fish were shipped as eyed eggs from Scully Creek, Lakelse Lake near Terrace, B.C. After hatching, the young were reared in circular or oval Fiberglas tanks at the Biological Station, Nanaimo, B.C. They were the progeny of a single cross in each of the 3 years. Subject to ambient water temperatures from a lake source, the fish were hand-fed on a diet of beef liver and Clark's pellet feed (J. R. Clark & Co., Salt Lake City, Utah) supplemented with a weekly supply of frozen brine shrimp. No history of epidemic disease, mortality in excess of 10%,[1] or poor growth was present in any of the stocks tested.

When transferred from the rearing tanks the fish were first screened for a fairly uniform, intermediate size (mean ±1 SD) and then distributed by random numbers to the experimental tanks (see Table 1, 3). The order of tanks according to ration was also assigned by random numbers.

Experimental Tanks

Twelve 197-liter (43-gal) oval tanks equipped with submerged jets to provide circulation and efficient water exchange were used (Fig. 1, 2; see also Alderdice et al., 1966). These were modified slightly by the addition of recirculating pumps and a gas-stripping column to supply approximately air-saturated water at each controlled temperature. In most instances a 12-inch, tubular air-breaker was provided as an additional source of oxygen, and served as an emergency stand-by. Water was flushed through the tanks at a rate of 240 liters/hr (53 gal/hr). At no time was the oxygen saturation found to exceed 106% or be less than 87%. The average was 95%. Cover was made available by a black coating over the center half of the Plexiglas top.

Water velocity within the tank ranged from 9 to 15 cm/sec (0.3–0.5 ft/sec) for most of the oval path; lower velocities occurred in the vicinity of the central drain. Fish were observed swimming mostly in the main flow away from the areas of reduced velocity. The application of a low velocity current was considered desirable not only for its cleaning action but also to facilitate food presentation and to maintain a greater uniformity of activity between tanks. Spontaneous activity is temperature-dependent in many fish including salmonids (Brown, 1957; Fisher, 1958). By inducing all fish to swim constantly reduction in this inherent variable was effected.

Temperature and Light

By cross-mixing between temperature-controlled supply lines with hand valves, or by use of thermostatic valves, temperatures were maintained within ±0.3 C (an average of ±2 SD for all tanks, read from standardized thermographs — see Table 1). The extremes of experimental temperature (1 and 24 C) were chosen to be approximately 1 degree C above and below the respective lower and upper lethal temperatures for underyearling sockeye in fresh water (Brett, 1952).

The A-group fish were transferred from culture tanks at an ambient temperature of 7 C to their assigned growth tanks. The temperature was raised at a rate of 1 degree C/day, and length–weight measurements were taken as soon as the prescribed temperature for all tanks was reached (June 2). Since a growth "adjustment" period of the order of 2 weeks was observed in the initial experiments (see Fig. 3), the B-group fish were provided with this further introductory period. Their ambient temperature ranged from 10 to 12 C. The fish tested at 1 C were allowed an additional 2 weeks of acclimation at this low temperature after an intermediate 2 weeks at 5 C.

Fluorescent lighting illuminated the tanks with a minimum of 15 ft-c at the water surface. This was controlled by a time switch set for a 9-hr day (0800–1700 hr, PST). Natural light was reflected on the tanks from a louvre-shaded window providing normal photoperiod from spring to early fall.

---

[1]From physical damage, nipping, popeye, and accidental loss.

TABLE 1. Tank temperature and vital statistics of A-group sockeye fed on excess ration. Data for 1 C have been included from B group. Variability is recorded as ±2 SE except for temperatures (±2 SD).

	Acclimation temperature (*C*)					
	1	5	10	15	20	24
Mean temp (*C*)	1.09±0.16	4.80±0.47	9.92±0.16	14.91±0.12	20.09±0.28	23.68±0.44
Test period (*days*)	83	219	219	220	224	44
Initial length (*cm*)	8.66±0.11	5.41±0.08	5.74±0.14	5.65±0.12	5.81±0.05	5.67±0.08
Initial wt (g)	6.04±0.22	1.18±0.06	1.43±0.10	1.33±0.09	1.40±0.08	1.31±0.06
Terminal length (*cm*)	9.20±0.12	10.14±0.68	16.78±1.14	19.73±0.86	16.85±0.47	5.74±0.20
Terminal wt (g)	7.69±0.30	7.83±1.76	46.40±10.50	85.50±12.70	51.70±4.70	1.18±0.22
Initial growth rate (%/*day*)	–	1.48±0.57	2.97±0.73	4.24±0.57	5.05±0.66	−0.34±0.35
Intermediate growth rate (%/*day*)	–	0.99±0.16	1.96±0.17	2.60±0.18	1.81±0.17	−0.20±0.66
Terminal growth rate (%/*day*)	0.23±0.06	0.76±0.11	1.35±0.12	1.42±0.11	1.21±0.13	(50% dead)

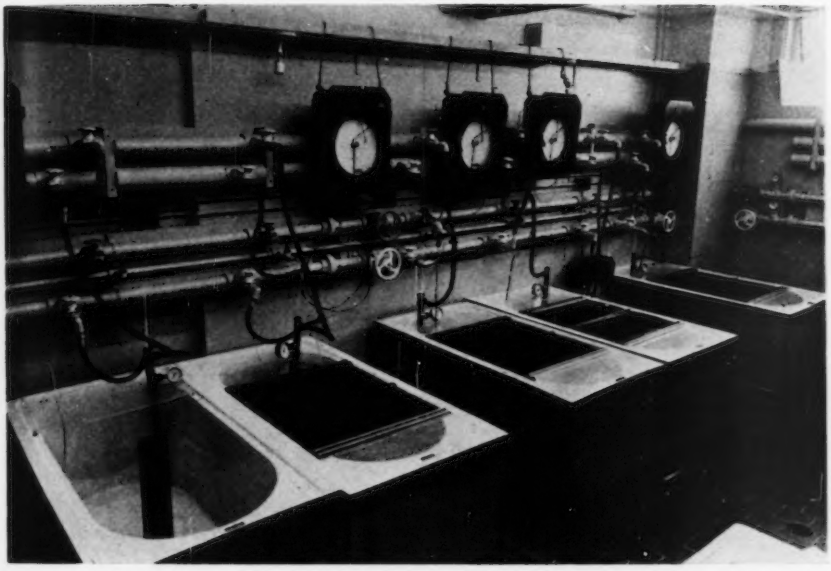

FIG. 1. Typical arrangement of tanks and services assembled for growth experiments.

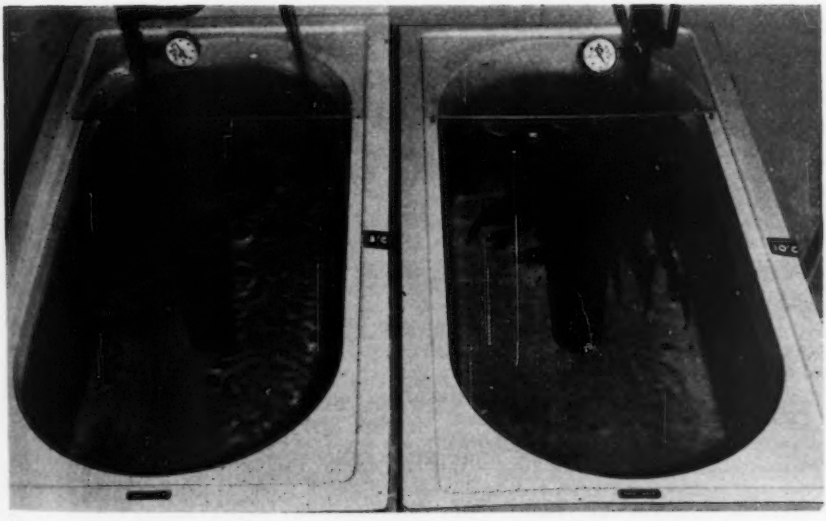

FIG. 2. Example of size difference in A-group fish after 3 months at 5 and 10 C, on excess ration. Note uniformity of size within tanks.

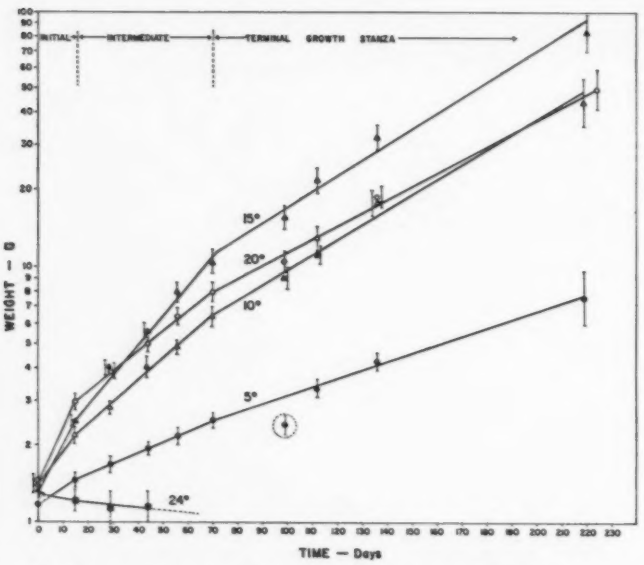

FIG. 3. Mean weights of samples ($n = 20$) of A-group fish held at five temperatures on excess rations. Experiments started on June 2. Limits are for ±2 SE. Three stanzas of growth are indicated according to inflection of slopes. One point (circled) for fish at 5 C was discarded as being beyond the 95% confidence limit of the mean growth rate.

SAMPLING PROCEDURE

A GROUP

Samples of 20 fish were removed every 2 weeks from an initial stock of 250 fish per tank. On each occasion fish were herded into one end of the tank and caught by drawing a small dipnet vertically through the group. The fish were killed by anaesthetic (150 ppm M.S. 222), and rolled in a slightly damp cloth to remove surface moisture. Fork length and weight measurements were taken to the nearest 0.1 cm and 0.01 g. The fish were then placed into a deep freeze for later analysis of gross body constituents. The possibility of sampling bias was tested by measuring all the fish in a control tank in lots of 20, throughout the experimental period. No significant difference occurred between the sample and remaining population in seven such comparisons ($P < 0.05$, using the method of Hubbs and Hubbs, 1953).

B GROUP

To reduce chance differences between samples within each tank, later experiments were conducted with 25 fish only. These were lightly anaesthetized for measurements every 2 weeks and returned immediately to their respective tanks. Fish were not fed on weighing days.

To check on the possible effect of periodic handling and anaesthetic all the fish in a tank of 250 were subjected to "standard treatment" once a month and their weight relations compared with a control group. No significant difference was apparent over a 3-month period although the anaesthetized group grew 18% faster in the first 2 months, then slowed down slightly. Because of the rapid recovery of the fish and their excellent appetite, it was assumed that the effect of the biweekly treatment was unlikely to be different from the monthly check.

FOOD AND FEEDING

Although essentially the same ingredients were used (see Appendix A) the composition of the diet differed between the two groups (Table 2). The moisture content was purposefully

TABLE 2. Composition and calorific value of diet fed to the two groups of fish. Figures obtained from an average of 10 samples (A group) and 14 samples (B group).

	A group (1964–65)		B group (1965–67)	
	Wet (%)	Dry (%)	Wet (%)	Dry (%)
Components				
Water	51	–	63	–
Protein	25	50	22	60
Fat	13	27	6	16
Ash	4	8	3	8
Fiber[a]	1	2	1	2
Carbohydrate[a]	6	13	6	14
Kcal/g				
Bomb calorimeter	–	–	2.0	5.4
Calculated[b]	2.4	5.9	2.0	5.5

[a]Approximate fiber content determined from constituents (Anon., 1959) with carbohydrate estimated by difference.
[b]Calorific value calculated from proportion of dry constituents using values of 5.7 (protein), 9.5 (fat), and 4.0 (carbohydrate) kcal/g.

increased from an average of 51% for A group to 63% for B group, for which controlled rations were used, to facilitate dispensing. The reduction in fat was the result of differences between years in the composition of the major ingredients — canned salmon and commercial pelleted feed. New food was prepared every 2–3 weeks as required and stored at –10 C, the moisture content being checked on each occasion. Proximate analyses of frozen spot-samples were performed at the end of the test period. The methods used were the same as those for analysing fish, described in the next section.

The diet is one that has evolved over a number of years for use in culturing experimental fish in our laboratory. Obviously high in nutrient quality it has provided excellent growth, good condition, and no mortalities. Since the difference in diet composition occurred *between* A and B groups, and not *within* the B group for which restricted rations were used, the change did not result in any significant difference between the various excess feeding regimes, although there is a tendency for the A group to have somewhat higher mean growth rates (see Fig. 6).

For the A group, food was dispensed by shredding frozen cubes through a hand ricer. The fish were fed to satiation three times a day at 4-hr intervals, starting at 0830 hr. This was termed "excess." Depending on temperature, the daily amount presented during the terminal growth stanza ranged from 10 to 14% of the body weight on a dry weight basis.

The B group was fed with a small press that ejected a prescribed weight in the form of multiple short strings of unfrozen food. The restricted rations selected as a fraction of dry body weight were: 0, 1.5, 3, 4.5, and 6%, and excess. It was discovered that a single ration of 3%/day, dispensed in about 5 min, was sufficient to allow each fish to obtain a "fair share" of the food. The 1.5% daily ration was therefore presented as 3% every 2 days, and the 6% ration as 3% twice a day. In the case of excess ration the uneaten particles were washed out of the tank within 3–8 min from the time of introduction. For the B group the small wastage involved was assessed, providing an estimate of the maximum daily intake.

Sockeye are a naturally schooling fish. As underyearlings[2] they do not display obvious aggressive or territorial behaviour when food is evenly dispersed and general uniformity of conditions prevail within holding tanks. If food is *not* consumed proportionately amongst all fish, growth depensation occurs, resulting in an increased size-differential with time (Magnuson, 1962). The variance, as indicated in the growth relations of A-group fish (log weight ±2 SE, $n = 20$, see Fig. 3), did not differ in extreme instances by more than 33% of the overall mean value, except at 24 C where growth was inhibited, and for the last weighing as a result of the smaller sample ($n = 10$). Less difference occurred in the B-group experiments with the exception of three instances where there was a change in variance associated with the accidental loss of fish under anaesthetic, and one instance where maintenance ration was involved. In general, disparity in consumption of food among the members of each sample did not occur to any significant degree.

Proximate Analysis and Calorific Value

At the beginning and end of each experiment gross body constituents were determined from samples of 20–25[3] fish, starved for 24 hr. Subsamples of five fish were ground to a fine homogenate and weighed-fractions were used to determine water, fat, and protein content in duplicate by the following methods.

Water — Approximately half the subsample was oven-dried at 105 C for 24 hr. A correction for any moisture loss during deep-freezing was applied.

From the sum of all samples taken in the 1964 tests and those at the start of the 1965 tests a mean water content of 74.4 ± 1.2% (±2 SE, $n = 24$) was obtained. The B group alone averaged 74.9%. As a basis for calculating the ration in terms of fractions of dry body weight a general level of 25% dry material was applied throughout.

Protein — Nitrogen content was determined by the micro-Kjeldahl technique outlined by the Association of Official Analytical Chemists (1960). The value obtained was multiplied by 6.25 to obtain the average protein value.

Nonprotein nitrogen — The difference between the acid-soluble and the acid-insoluble nitrogen values (method of Association of Official Analytical Chemists, 1960) gave the nonprotein nitrogen content. It averaged 0.2% of dry weight (about 0.05% of wet weight) and varied from an undetectable amount to 1.2% of dry weight. The highest levels were associated with the highest temperatures, independent of ration except for starved fish, which contained consistently low levels. Since only major constituents were being examined these small values have been omitted from the compilation in Table 3.

Fat — Lipid analyses were first performed by the chloroform–methanol wet extraction technique of Folch et al. (1957). This was later changed to hexane extraction from dried material (see Appendix B). A slight difference in values was ascribed to the possible carry over of moisture by the wet extraction method. An appropriate correction was therefore applied to bring all results to a common basis for dry extraction.

Carbohydrate — No analysis of carbohydrate was conducted since this constituent does not amount to more than 0.5% of body weight (Vinogradov, 1953; Black, 1958). Highest concentration occurs in the liver where values from 3 to 4% have been obtained for normal feeding fish (Hochachka and Sinclair, 1962).

Calorimetry — Calorific values were determined with a Parr bomb calorimeter (methods prescribed by the American Society for Testing Materials, 1966).

When the calorific content was calculated from proximate analysis, values of 9.5 kcal/g for fat and 5.7 kcal/g for protein were applied (Brody, 1945; Kleiber, 1961).

Data Compilation and Analysis

The quantitative laws governing growth have been treated as general concepts by such authors as Bertalanffy (1957) and Needham (1964), with more specific consideration of the process

[2]Aggressive behaviour has been observed in yearling fish, with intense periods occurring soon after feeding.

[3]Except in a few cases listed in Table 3.

TABLE 3. The effect of temperature and ration on the weight, growth rate, calorific value, and body constituents of sockeye fingerlings at the beginning ("cultured" fish) and end of each experiment.

TABLE 3. The effect of temperature and ration on the weight, growth rate, calorific value, and body constituents of sockeye fingerlings at the beginning ("cultured" fish) and end of each experiment.

Temp (C)	Ration level (% *dry wt*)	No. fish (*n*)	Initial wet wt (g)	Terminal wet wt (g)	Growth rate (%/*day*)	Duration (*days*)	Duration (*day-degrees*)	Calories *Kcal/g*)	Water (%)	Protein (%)	Fat (%)	Total[a] (%)
1	Cultured	25	6.01	–	–	Start	–	6.26[b]	74.2	15,8	7.6	97.5
	0.0	25	5.62	4.96	−0.14±0.06	83	83	6.01[b]	80.8	14.6	3.4	98.8
	1.48	25	6.19	7.19	0.17±0.08	83	83	6.54[b]	77.2[c]	14.9[c]	6.6[c]	98.7
	3.03[d]	24	6.04	7.69	0.23±0.06	83	83	6.21[b]	76.2	13.4	7.5	97.1
5	Cultured	25	5.52	–	–	Start	–	5.94	75.4	15.4	6.3	97.1
	0.0	25	5.62	5.16	−0.21±0.12	99	495	5.25	80.3	15.1	1.9	97.3
	1.78	24	5.43	7.26	0.38±0.11	99	495	6.16	75.4	14.2	7.4	97.0
	3.44	25	5.79	10.10	0.67±0.09	99	495	6.59	72.1	17.3	9.1	98.5
	3.59[d]	24	6.30	10.65	0.64±0.09	99	495	6.63	72.6	16.3	9.5	98.4
	Excess	21	5.97	9.92	0.61±0.10	99	495	6.35	72.6	16.0	8.9	97.5
10	Cultured	25	6.02	–	–	Start	–	5.94	75.4	15.4	6.3	97.1
	0.0	19	5.24	3.83	−0.41±0.10	99	990	5.10	82.0	14.1	1.3	97.4
	1.77	23	6.14	8.04	0.31±0.10	99	990	5.95	77.3	14.0	5.9	97.2
	3.42	23	6.58	12.95	0.80±0.12	99	990	6.24	75.1	16.6	6.5	98.2
	4.99	20	7.36	20.04	1.17±0.14	99	990	6.23	72.9	19.0	6.6	98.5
	4.93[d]	25	7.51	20.37	1.17±0.13	99	990	6.19	71.7	15.9	9.0	96.6
15	Cultured	25	6.51	–	–	Start	–	5.94	75.4	15.4	6.3	97.1
	0.0	14	5.61	3.30	−0.64±0.16	99	1485	4.62	83.2	13.2	0.6	97.0
	1.87	10	5.90	6.39	0.10±0.13	99	1485	5.42	75.7	16.0	4.4	96.1
	3.51	19	7.43	12.82	0.67±0.12	99	1485	5.96	74.9	18.6	5.2	98.7
	4.85	21	7.30	18.99	1.17±0.09	99	1485	6.24	72.2	20.0	6.0	98.2
	6.08	21	7.69	25.45	1.44±0.14	99	1485	6.33	71.8	17.3	8.5	97.6
	Excess	19	8.75	29.28	1.26±0.19	99	1485	6.37	71.3	19.7	7.6	97.6
20	Cultured	25	6.78	–	–	Start	–	6.26[b]	74.2	15.8	7.6	97.5
	0.0	25	5.92	3.27	−0.50±0.08	85	1700	4.80[b]	86.9	9.4	1.0	97.3
	1.52	24	6.30	5.09	−0.19±0.10	85	1700	5.53[b]	81.6	12.7	3.1	97.4
	3.00	23	6.70	7.01	0.08±0.10	85	1700	5.81[b]	79.2	13.2	4.8	97.2
	4.36	10	7.18	11.32	0.48±0.09	85	1700	6.47[b]	77.2	14.7	6.7	98.6
	5.65	16	7.93	18.45	0.84±0.07	85	1700	6.39[b]	75.1	13.6	8.6	97.3
24	Cultured	20	1.39	–	–	Start	–	6.10[b]	76.5	16.5	5.2	97.2
	Excess	20	1.31	1.18	−0.19±0.35	44	1056	5.04[b]	79.4	14.9	2.0	96.3

[a]Ash was not determined. The mean amount by difference = 2% of the wet weight, allowing for a small percentage of carbohydrate and non-protein nitrogen.

[b]Calculated calorific content; otherwise obtained by bomb calorimetry.

[c]±2 SE for $n = 25$; water = ±0.70; protein = ±0.74; fat = ±0.51.

[d]A higher ration was prescribed but the fish did not accept more than recorded.

in fish provided by Brown (1957), Taylor (1962), and Winberg (1956). Under conditions of environmental control and in the absence of limiting factors, growth is a multiplicative process, which, in the early stages of life, often follows an exponential curve. Under the influence of apparent inhibition with age its overall configuration tends to follow the sigmoid shape of a logistic curve. The instantaneous rate of gain in weight per unit weight (per cent change in weight/time, or specific growth rate) declines progressively with increasing size.

The general equation for exponential growth is $w = be^{kt}$, where w is size and t is time. When $\log_e w$ is plotted against t, (Fig. 3) the slope $k \times 100$ is equivalent to the specific growth rate (G). This was determined by taking length–weight measurements every 2 weeks, usually for a 10- to 12-week period. An estimate of the error of k was computed from the pooled data of all measurements (±2 SE). The restricted ration was adjusted at the *start* of each biweekly period according to the new mean weight obtained, on a dry weight basis. The *actual* proportion of the ration was subsequently calculated according to the observed mean weight achieved by the fish during the biweekly interval, e.g., as the fish grew the actual fraction became slightly less than the ration initially prescribed.

Because of the progressive change in growth rate that appears to occur in stanzas of decreasing slope (Fig. 3), a period of relatively stable growth from October to February (Fig. 5) was accepted as a suitable time for making comparisons between treatments, spread over a number of years.

RESULTS

Temperature × Excess Ration — A Group

As a result of the favorable change in density, diet, and feeding frequency that accompanied the transfer from culture to experimental conditions, all stocks showed a marked increase in growth rate, except those at 24 C (Fig. 3, Table 1). This surge declined after an initial 2 weeks "adjustment" (possibly less) and the growth rate remained constant for the next 8 weeks. At this time (August 10) a synchronous decrease in slope occurred in all stocks despite the difference in acclimation temperatures of 5–20 C. No further change was noted up to the time of terminating the experiment 21 weeks later (January 12). Such seasonal variations in growth rate have been observed by Swift (1955) for hatchery-reared brown trout (*Salmo trutta*) exposed to normal temperature and light. A reduced growth rate occurred in May and June despite increasing temperature and daylight. It is possible that changes in the production of growth hormone could account for such temperature-independent decreases in growth.

The maximum growth rate recorded in the initial period was 5.05%/day at 20 C, a doubling of weight in 2 weeks. The relation between temperature and maximum growth rate for the two subsequent stable periods ("intermediate" and "terminal" — Table 1) has been plotted in Fig. 4 including one instance where excess ration was fed in the B-group experiments (1 C, Table 3). At an age of 5–7 months, from the start of feeding, a pronounced optimum temperature for growth occurred at 15 C. Although still apparent for 7- to 12-month-old fish, the peaking of the relation is sufficiently reduced that no statistical difference exists between the growth rates at 10 and 15 C. With increasing age, growth continues to slow down so that optimum temperatures become progressively less apparent. The expression of maximum growth rate in relation

to acclimation temperature (Fig. 4) has been called the "scope for growth" by Warren and Davis (1967) by analogy with the "metabolic scope," which Fry (1947) used to describe the capacity to elevate metabolic rate above the maintenance or standard level at various temperatures.

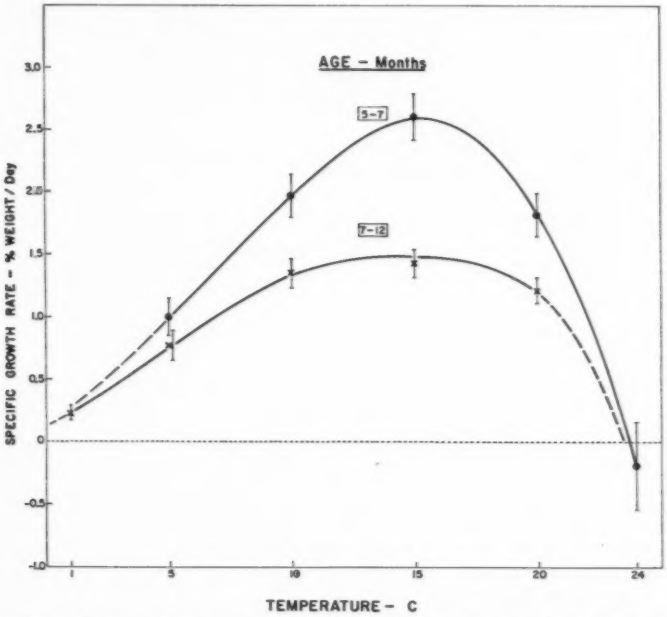

FIG. 4. Relation between temperature and growth rate (±2 SE) of young sockeye salmon fed on excess ration. Age is in months from start of feeding.

At 24 C some of the young salmon would not accept food. Mortalities commenced in the 1st week. The mean weight declined and the variance increased as a consequence of the variability in feeding response. Within 3 weeks one-third of the stock were "pin-heads," which died within the month. By the 44th day over 50% were dead, at which time the experiment was terminated.

Similar problems of feeding and subsequent mortality near the boundary of the upper tolerance limit for this species have been observed by Donaldson and Foster (1940) at 23 C, and were encountered by Brett (1952) when he attempted to acclimate 3-month-old sockeye to high temperatures, elevated by stages to 24 C.

TEMPERATURE × PRESCRIBED RATION — B GROUP

An example of the effect of ration level on growth rate at a given temperature is illustrated in Fig. 5. It was characteristic of most of the data to exhibit

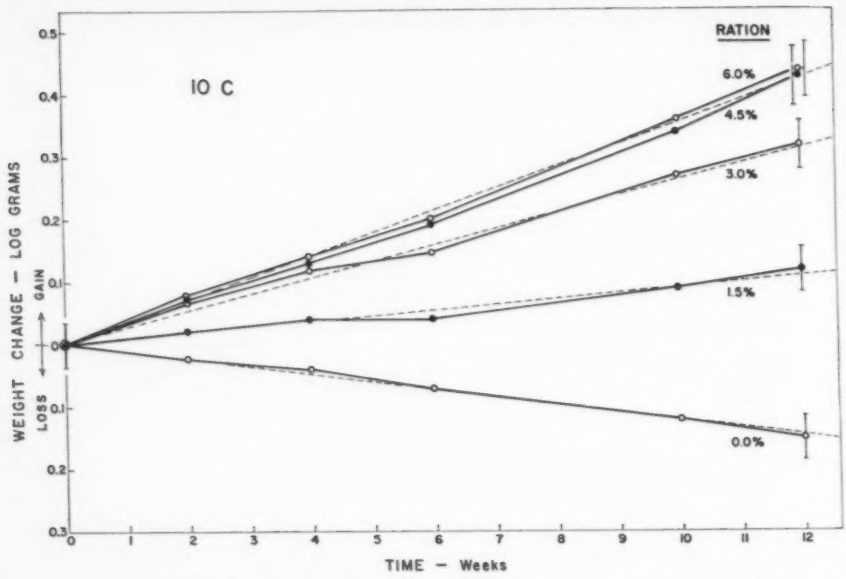

FIG. 5. The effect of ration on growth rate of fingerling sockeye at 10 C. For ease in comparing rates (slopes) a common origin for all stocks has been used. Solid lines link each mean weight ($n=25$; limits $=\pm 2$ SE). Broken lines represent the computed slopes for all data at each ration. Prescribed rations are indicated; actual consumed rations are presented in Table 3. Note that the statistical separation of the fish on 3% ration was not possible in less than 6 weeks.

deviations along the path of exponential growth, and these deviations, together with the difference in weight within each lot of 25 fish, contributed to the variance in slope (specific growth rate, Table 3). At high feeding rates, approaching the maximum intake, it is apparent that the time required to demonstrate a significant difference in growth rate between two levels of ration was of the order of 6–8 weeks (e.g., 3 and 4.5% ration at 10 C, Fig. 5).

When growth rate was plotted against ration for each temperature a series of curves was obtained, which change in form from a "logistic shape" at 20 C to a "geometric shape" at lower temperatures (Fig. 6). In the absence of a generally suitable transformation a smooth curve has been fitted by eye to pass through the means ± 2 SE. Since various growth parameters can be obtained by interpolation all the computed points are presented graphically for inspection in Fig. 6.

The parameters of particular interest were: (1) the maintenance ration, i.e., that ration that just maintains the fish without any weight change; (2) the optimum ration, i.e., that ration that provides for the greatest growth for least intake (most efficient); and (3) the maximum ration, i.e., that ration that just provides for the maximum growth rate.

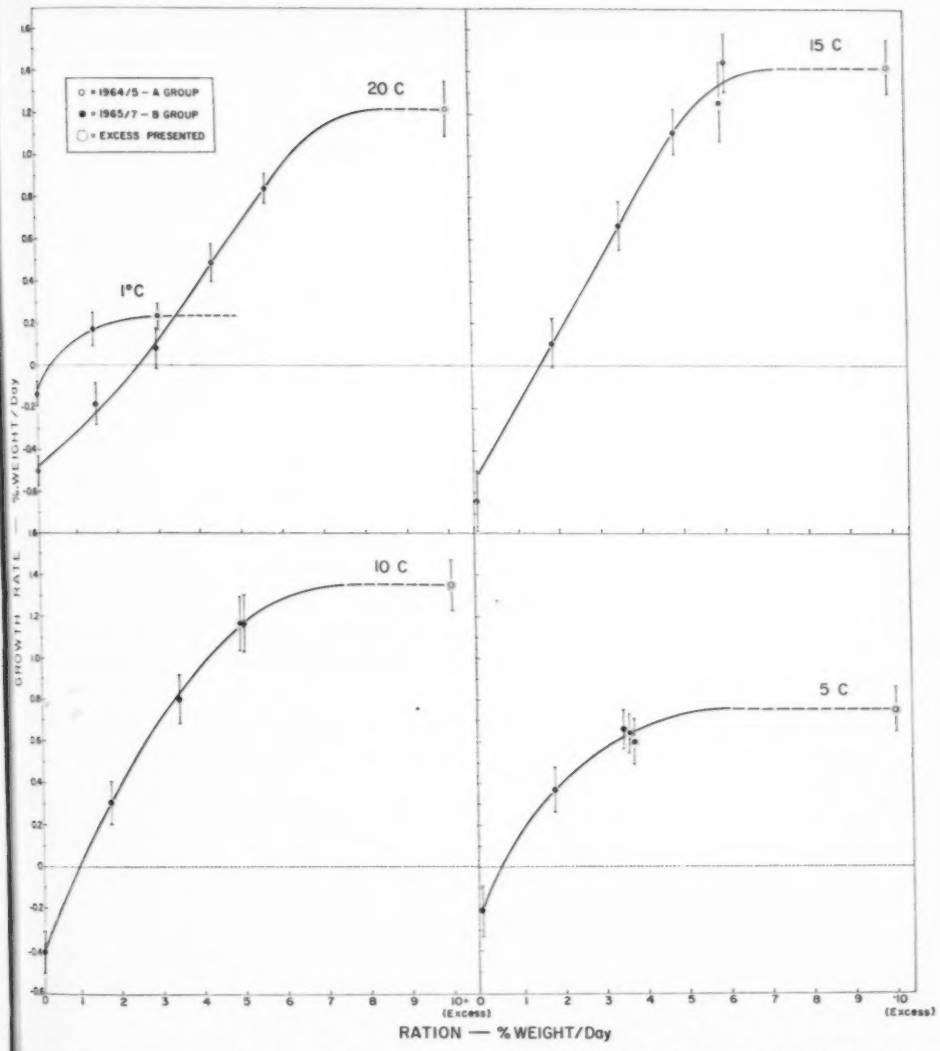

FIG. 6. Relation of growth rate to ration at five temperatures. Points for A-group fish (○) have been plotted for an average ration of 10%, presented as excess. Where B-group fish were satiated by the prescribed ration, or fed a measured excess, the points have been circled.

These may be derived geometrically (Fig. 7) (cf. Thompson, 1941). The fact that the optimum ration can be determined by drawing the tangent to the curve from the origin may be proven by calculating the ratio of *growth rate* to *ration* for a series of rations. The parameter most difficult to determine

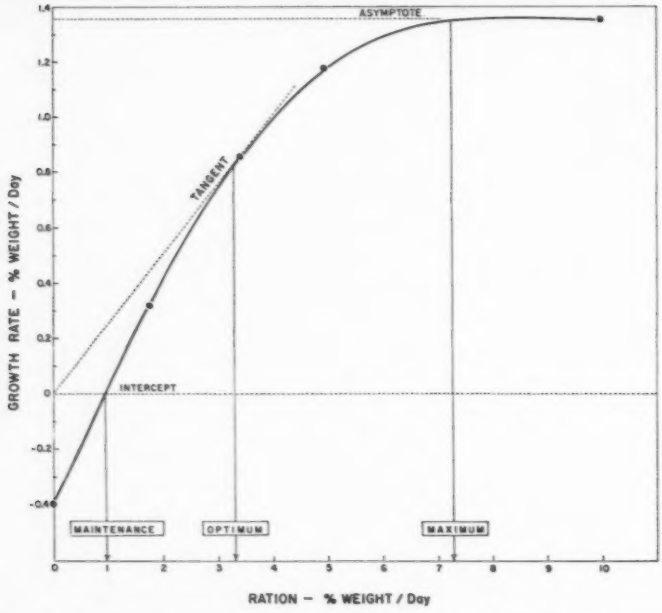

FIG. 7. Geometric derivation of various parameters of growth with accompanying ration, using the data for fish at 10 C as an example.

accurately was the maximum ration, involving the asymptote for maximum growth rate, the values for which were subject to greater variability than most of the other rates.

With increasing temperature there was a large increase in the ration required to meet the defined growth parameters (Fig. 8). The maintenance ration at 20 C was seven times that at 1 C; the optimum was five times greater for the same temperature difference; and the maximum just under three times greater. The maximum food intake per day was approximately 8% of the dry body weight at 20 C. The temperature × ration relations could be made linear (Fig. 8, equations), improving the basis for estimated values and providing for extrapolation to the extremes of temperature at which food was still accepted. Above 23.3 C loss of appetite and inefficiency of food conversion were responsible for the breakdown in the overall relation, and the ultimate death of the fish.

GROSS BODY CONSTITUENTS

Both ration level and temperature had a considerable effect on all body constituents (Table 3). They ranged from 86.9% water, 9.4% protein, and 1.0% fat at 20 C for fish starved for 83 days, to 71.3% water, 19.7% protein, and 7.6% fat for fish on an excess ration for 99 days at 15 C — the optimum

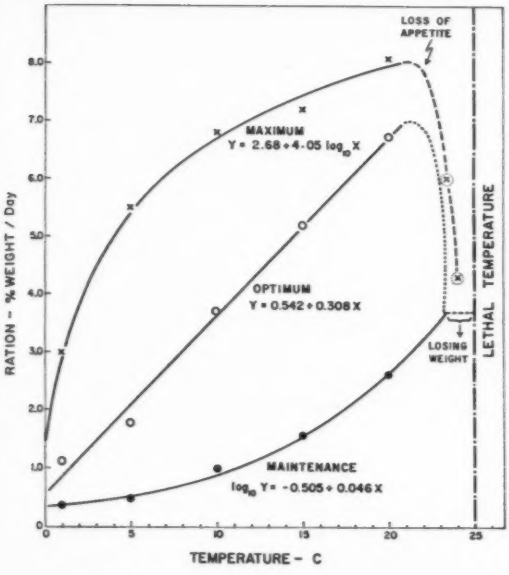

FIG. 8. Relation of maintenance, optimum, and maximum rations to temperature. Points were determined as outlined in text, except the two circled points, which were derived by inspection from Fig. 11.

temperature for growth. This dynamic and variable state of body constituents is apparent for all stocks and is represented by the progressive change in moisture content depicted in Fig. 9. At all temperatures a lower water fraction occurred with increasing ration, reaching a minimum of about 72% by the termination of the experiments.

Among the starved fish the moisture content varied according to temperature, with the exception of the fish held at 1 C. Since the rate of change in composition will be dependent on some function of temperature and time, it would be expected that changes would proceed very slowly at 1 C. Comparison between experiments of equal duration cannot be made on common physiological grounds unless a static state of body composition has been reached. This is suggested by including a column in Table 3 for day-degrees, as one indication of physiological time. It may be seen that on this basis the experiments ranged from 83 to 1700 day-degrees. In addition it was an inevitable outcome that considerable difference in size would characterize the various groups at the terminal stage. Size has been shown to influence body composition of young pink salmon, *Oncorhynchus gorbuscha* (Parker and Vanstone, 1966).

As water content increased, both fat and protein decreased.[4] In the light of this highly significant negative correlation (fat = -0.89, $P < 0.01$; protein = -0.80, $P < 0.01$) it was possible to derive simple equations with appropriate

[4]Above the maintenance ration any decrease in protein is strictly relative; however, fat may show some absolute changes.

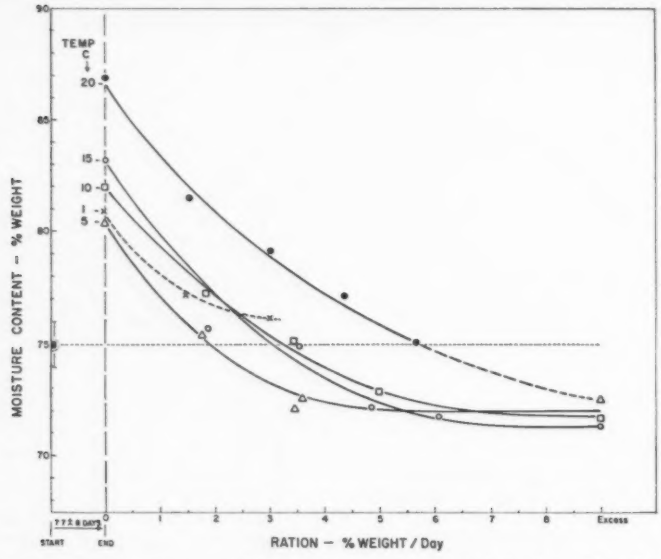

FIG. 9. Moisture content of fish at the start (single circled point with ±2 SE) and end of growth experiments. Each "eyed" curve represents the final percentage of body water in relation to daily ration for a given temperature. With the exception of 1 C, it is likely that these represent equilibrium states for any ration and temperature.

confidence limits relating water content to corresponding fat and protein levels (Fig. 10). Contributing to the variability in composition between samples of fish are the direct and interacting effects of temperature, ration, size, growth rate, and conversion efficiency, coupled with a degree of curvilinearity produced by approaching threshold levels of these major constituents. No attempt has been made to sort these out. It is apparent, however, that by determining the moisture content *only* the total gross body constituents can be estimated fairly accurately from the above relations, assuming an average ash of 2% of wet weight (Table 3).

DISCUSSION

SOURCES OF ERROR

The experiments were designed to provide statistical sensitivity for determining the difference *between* treatments (temperature and ration) by reducing the variability *within* lots (tanks of fish). Reduction in genetic variability was achieved by selecting a limited size range from the progeny of one female. Although this was undoubtedly an effective approach in any one year, it did not anticipate the subsequent need for comparison between years, as well as the desirable feature of obtaining a wider representation of the population. The limited gene pool can be expected to be one of the factors

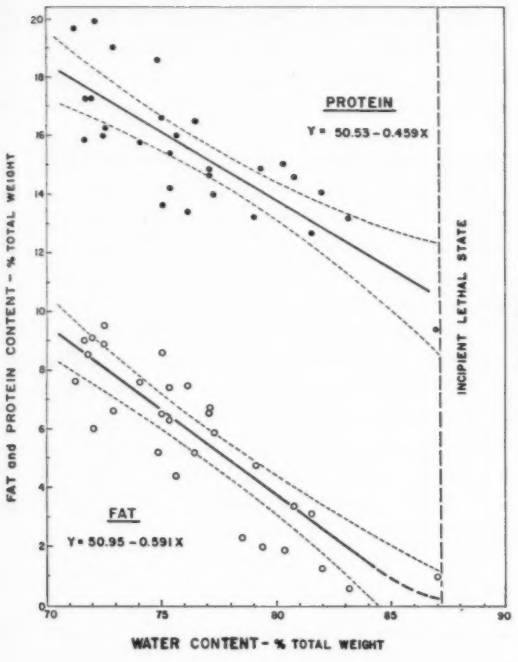

FIG. 10. Relation of protein and fat content to water content of young sockeye. Points represent pooled data from all temperatures and rations. Lines of best fit determined by least squares, with 68% confidence limits (broken lines).

ontributing to the differences in maximum growth rate between years, as exemplified by the various stocks of fish on excess ration at any one temperature (Fig. 6). Yet it can be seen that the dissimilarity was not great, amounting, for example, to a difference in specific growth rate of about 0.2%/day for stocks at 15 C. Since some change in diet occurred between A and B groups (Table 2), and it was decided to present excess food twice a day rather than three times a day in later experiments, the question of maximum growth rate for the species at this age and size remains undetermined though probably not seriously underestimated. Indeed, with advances in nutrition and in making environmental conditions optimal, it is doubtful if the full potential for growth of any species including man has yet been assessed.

It is apparent from the change in body constituents that accompanied the different treatments that the presentation of restricted rations on the basis of the initial moisture content would apply throughout the experimental period only to those instances that did not deviate significantly from the original state (signified by the points close to the dotted line for 75% moisture in Fig. 9). Except for starving and excess-fed fish an error progressively creeps in for all other instances, reaching a maximum at the end of the test time. The extremes occur at low temperature on high ration, and at high temperature on low ration. For example, the fish held at 5 C on 3.5% ration had a terminal

average moisture content of 72.5%. On the final day the prescribed ration of 3.5% was actually 3.2% of the existing dry weight. Lacking knowledge of the rate at which the constituents changed, coupled with the fact that it would apply in varying degrees up to the sort of error indicated, no correction was possible.

The basis of conducting exacting experiments on bioenergetics and growth in fish is treated with insight by Warren and Davis (1967). From a bioenergetic point of view the conversion of food should be based on calorific rather than dry weight values because of the differences in the fat/protein ratio. When considering growth only, there is justification for calculations based on nitrogen content such as Gerking (1962) used in studies on food conversion of the bluegill, *Lepomis macrochirus*. Since the protein fraction of the dry weight varied somewhat less than the fat fraction (Fig. 10), the inherent error indicated above would be reduced accordingly. It is worth stating here that subsequent experiments are being conducted by increasing the number of fish to allow for biweekly subsamples to be withdrawn for appropriate analysis.

With the above limitations set forth it is possible to examine the combined effects of temperature and ration on the specific growth rate, and to proceed to a consideration of the efficiency of food conversion.

Temperature–Ration Relations

From the relation between ration and growth rate for each temperature (Fig. 6), the form of the growth × temperature curves for each prescribed ration may be determined (Fig. 11). The temperature for optimum growth shifts progressively to the left as the ration is reduced, moving from 15 C on excess ration to approximately 5 C for a ration of 1.5%/day. The outer curve is the same as that describing the scope for growth for 7- to 12-month-old sockeye presented previously (terminal growth rate, Table 1, Fig. 4). With the exception of the relation for starved fish, each of the remaining domed curves represents the scope for growth characterizing each reduced level of available food. As the fish grow older there would be a corresponding flattening of this fleet of curves, approaching the ultimate minimum growth rate for aged fish.

The shunt in optimum temperature supports the hypothesis that, for acclimated fish, digestion and growth can still proceed effectively at low temperatures. It suggests that either the enzymes involved are relatively temperature-independent (wide spectrum), that a fleet of enzymes is present with a wide range of temperature reactivity, or that rapid changes in the metabolic system occur to meet the new situation. The marked increase in energy requirements for maintenance that accompanies rising temperature (Fig. 8) accounts for the decreasing scope for growth, to the point of losing weight at low rations (Fig. 11).

Fish feeding on a limited ration in nature would achieve better growth by moving along the temperature axis, towards the appropriate optimum.

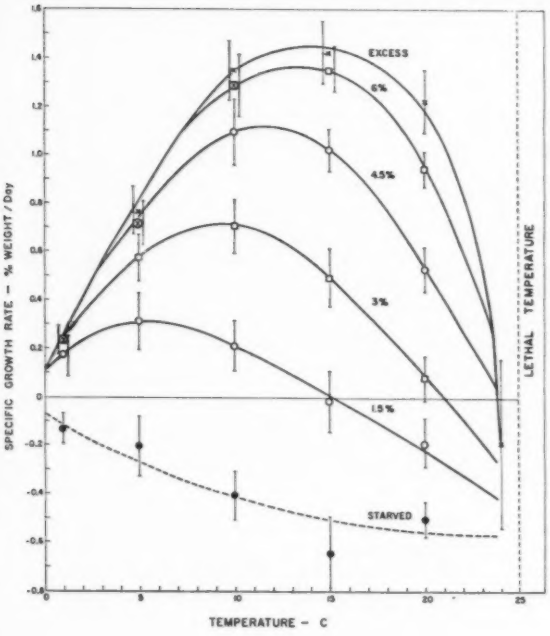

FIG. 11. Effect of reduced ration on the relation between growth rate (±2 SE) and temperature, for 7- to 12-month-old sockeye. Points for excess ration or where a prescribed ration turned out to be excessive are marked with an ×. The broken line for starved fish is a provisional interpretation.

Thus, at 15 C on a ration of 1.5%/day no growth would occur, whereas for the same ration at 5 C a size increase of 0.3%/day would be possible. The daily vertical movements of young sockeye observed during the summer in Babine Lake (Narver, MS, 1967) involved changes of 10 C, from near-surface feeding at dusk followed by a gradual retreat to depths of 100 ft or more shortly after dawn. This could be conceived as a mechanism evolved to improve growth in the presence of a limited food supply. Since yearling migrants frequently average no more than 6 g (Johnson, 1965), whereas experimentally fed young sockeye under optimum conditions may weigh as much as 80 g 4 months prior to the smolt phase, it is not hard to conceive that food is a limiting factor for this species in many lakes.

The predictive value associated with established food requirements is best illustrated by calculating the growth-rate isopleths (Fig. 12). The food ration necessary to provide for any given growth rate at any particular temperature may be ascertained readily by checking the appropriate combination. Since this applies to only one growth stanza, or age, it is possible to see that by a series of such experiments, conducted at selected intervals throughout the life history, a method of computing the total food requirements would be obtained.

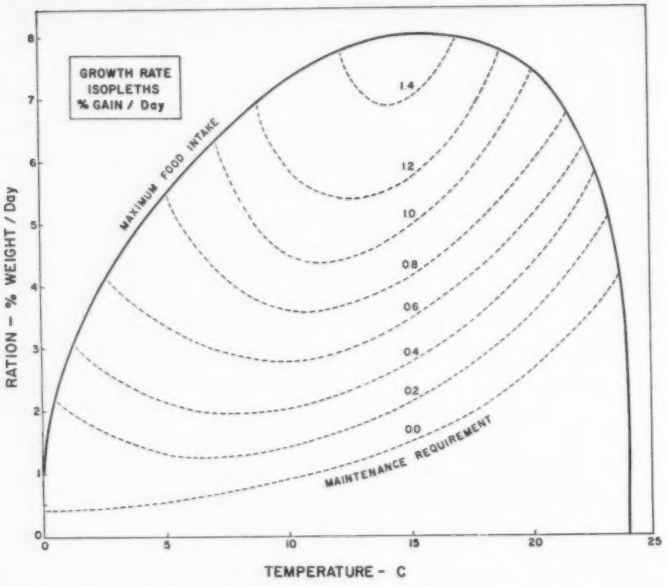

FIG. 12. Growth rate isopleths for yearling fingerling sockeye, showing the percentage gain in weight per day that would be expected for any combination of ration and temperature.

STARVATION EFFECT

The rate of loss of body weight for starved fish in relation to temperature was difficult to interpret since the mean rate for 15 C (−0.64%/day) was unexpectedly greater than the mean for the 20 C (−0.50%/day). These bordered on being statistically different ($P = 0.04$). A provisional line was drawn in Fig. 11 to represent the most likely relation for increased temperature.

The experiments for temperatures of 1 and 20 C were conducted a year later than those for 5, 10, and 15 C. Aside from a difference in the origin of stocks it was conceivable that spontaneous activity might have been highest at 15 C resulting in a generally higher metabolic rate than for the stock at 20 C. It should be noted, however, that the fish at 20 C had a terminal moisture content of 86.9%, whereas the fish at 15 C were 83.2% water (Table 3). A check on the provisional interpretation was made by conducting a comparable experiment at temperatures of 5, 10, 15, and 20 C for 12 weeks in 1967–68. At this time only fish of approximately twice the weight were available (12.0 ± 0.3 g). The results gave confirmation for the rate of loss of weight at 5 and 20 C but not for the intermediate points (Fig. 13).

It must be concluded that greater variability is either natural or somehow induced during starvation experiments. The sample size should therefore be increased and the conditions governing excitation should be rigidly controlled,

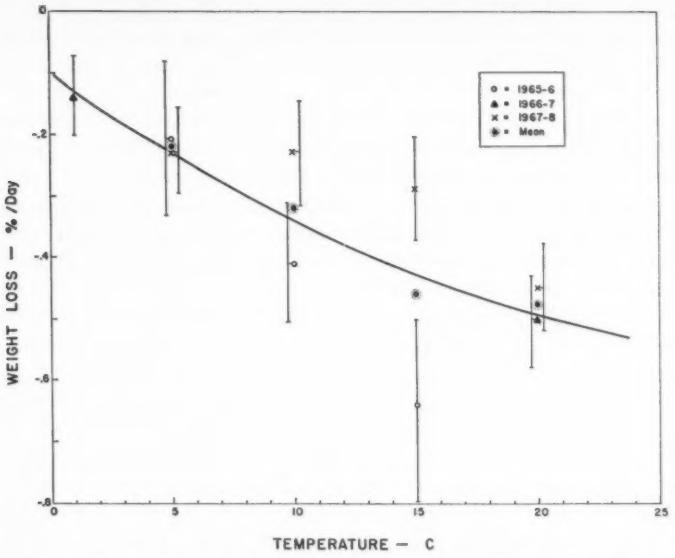

FIG. 13. Rate of loss of weight for starved fish in relation to temperature. Experiments conducted with three groups of fish in separate years. Initial mean weight of fish for experiments in 1965–67 was 6.5 g; that for 1967–68 was 12.0 g.

particularly regarding disturbances that may result inadvertently from the feeding of adjacent tanks. The mean for all data has been used in Fig. 13 as the best representation of the general temperature relation.

CONVERSION EFFICIENCIES

GROSS EFFICIENCY

Few studies of growth relations in fish have been performed with sufficient accounting of food consumed and flesh produced to permit determining conversion efficiencies (Kinne, 1960). With a common unit of dry weight, nitrogen content, or calorific value, the gross efficiency (E_g) may be calculated as the ratio of output to input by the formula $E_g = \frac{G}{I} \times 100\%$, where G = growth, and I = food intake. This simple index provides one of the most revealing aspects in analyzing growth phenomena, not only by indicating the circumstances under which the animal is most effective but also by providing a measure of the most economical use of food.

In addition to the factors of temperature and salinity, gross efficiency has been shown to depend on the type of diet, the feeding interval, and the size or age of the fish (Paloheimo and Dickie, 1966b; Pandian, 1967a, b). Conversion values range from 8% for omnivorous adult carp feeding on detritus

and algae (Kevern, 1966) to 44% for carnivorous young *Ophiocephalus striatus* feeding on a diet of prawns and chopped fish (Pandian, 1967a). Ivlev (1945) considered 35% to be a maximum for young fish on a high ration. Underyearling cutthroat trout, *Salmo clarki*, showed an individual variation of 13–37% in ability to convert a diet of housefly larvae and adults at a mean temperature of 8.5 C (Warren and Davis, 1967).

The combined effect of temperature and ration provided a small maximum area of 25% efficiency for fingerling sockeye, with a graphically determined center at 11.5 C and ration of 4.0%/day (Fig. 14).[5] The mapped isopleths for

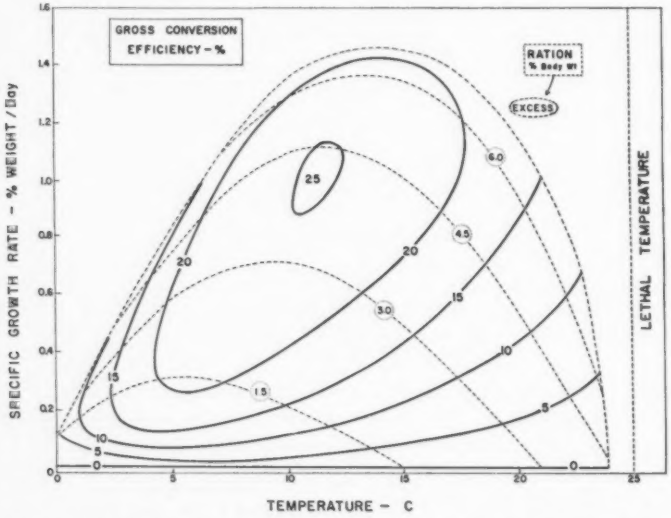

FIG. 14. Gross efficiency of food conversion in relation to temperature and ration, drawn as isopleths overlying the growth curves of Fig. 11 (broken lines).

decreasing efficiencies radiate outwards, with a "favourable axis" passing from the lower left corner diagonally upwards through the various growth optima for each prescribed ration. In line with this axis a large oval area is encompassed by the 20% conversion contour.

It is apparent that temperature may have as much effect as ration on conversion efficiency when the full ranges of both are considered. The conclusion of Winberg (1956) that temperature has virtually no effect appears to result from a lack of available evidence for temperatures *above* the optimum. When food is present in abundance (ad libitum, or excess rations), temperatures

[5]As an aid to interpreting this graph it is worth pointing out that by following a vertical line at any one temperature, say 10 C, the conversion efficiency increases from zero at a maintenance ration, passes through a maximum value at an intermediate ration, and then decreases reflecting some wasteful gorging at high rations (cf. Fig. 7).

below the optimum are associated with reduced food intake resulting in a fairly constant conversion efficiency, such as that along the "favourable axis" of Fig. 14. This interaction produces an apparent temperature independence, which proves to be circumstantial when the whole phenomenon is examined.

A similar sort of limitation appears to apply to the analyses of Paloheimo and Dickie (1966b) who concluded that at a given temperature increasing ration results in a decreasing conversion efficiency. Because of the paucity of available data on restricted rations the majority of instances cited by these authors relate to supraoptimum rations, which inevitably result in lowered efficiency. The full range of response is clearly demonstrated in the case of young sockeye by applying the method of analysis of Paloheimo and Dickie (1966b) who determined that the logarithm of the gross growth efficiency (log K_1) decreased linearly with increasing ration. Evidence to support such an hypothesis could come only from rations that were sufficiently above the maintenance level to provide for "excessive feeding." Efficiency of food assimilation increased from 71.5% to 86.5% for goldfish fed restricted rations near the maintenance level, at 21.5 C (Davies, 1963). Assuming a moisture content of 75% for the adult goldfish the respective rations ranged from about 0.5%/day to 2%/day on a dry weight basis. It is possible that in some species the position of maximum growth efficiency may occur at comparatively low rations, particularly under experimental conditions where the work associated with food-finding is minimal. As is indicated for young sockeye (Fig. 15) the inflection of log K_1 to a negative slope occurred at progressively higher rations with increasing temperature. An optimum ration was not even reached at 20 C. The latter circumstance prevails because fish would not accept a sufficiently high ration at this temperature to surpass the point of maximum conversion.

The small size of most yearling sockeye at the time of migration makes it highly unlikely that supraoptimum food conditions characterize this phase of life so that the use of log K_1 as developed by Paloheimo and Dickie would not be applicable during freshwater growth.

NET EFFICIENCY

If the maintenance ration is known a further calculation may be made of the net efficiency (E_n) by subtracting the fraction of the total ration that is involved in maintenance (M), thereby deriving the efficiency of utilization of the fraction of food available for growth. In this instance $E_n = \frac{G}{I-M} \times 100\%$.

Because Brown (1957) reported that the maintenance ration was difficult to determine directly, since the fish kept adapting their growth rate to compensate for reduced rations, Pandian (1967a) dismissed the value of determining net efficiency. Paloheimo and Dickie (1966b) also considered that the difficulties of obtaining satisfactory maintenance estimates precluded useful application of net efficiencies. However, since it has been shown that where stable growth

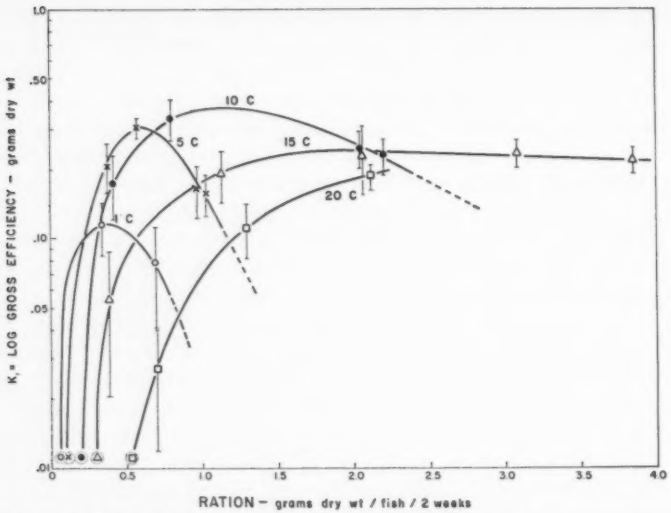

FIG. 15. Relation between logarithm of gross growth efficiency and ration at five different temperatures. Circled points were calculated from interpolated values (Fig. 6). The efficiency, K_1, was determined in the manner of Paloheimo and Dickie (1966b) using the daily gain in dry weight/daily dry ration. Straight line projections beyong the optimum suggest the relation determined by these authors.

Each point was calculated from the total growth during a 10- or 12-week period. Variability within tanks is indicated as ±1 SD of the biweekly K_1 values, which did not show any consistent trend in relation to increasing ration. Variance increases as K_1 approaches zero.

rates have been established the maintenance ration can be determined quite accurately by interpolation (see Fig. 6 and 8; also Warren and Davis, 1967), the case for net efficiency is worth further consideration.

Brody (1945) recounts that net values were used primarily to compare the efficiencies of farm produce (such as milk and eggs) without the maintenance costs for the different domestic animals being involved. Since it is a matter of producing flesh or converting energy in fish that is of concern it might seem reasonable to dismiss the net efficiency. However, it is important wherever possible to partition the energy relations to distinguish the relative contributions when comparing produce, species, rations, ages, or the effects of environmental factors.

Since the estimated maintenance portion of the ration is subtracted from the total ration, it is obvious that the net efficiency will always be greater than the gross efficiency. This difference is least at maximum growth rate on a high ration and greatest approaching the maintenance level, i.e., the utilization of that small fraction of the ration in excess of maintenance tends to be very efficient. By using actual and interpolated values from the curves relating growth rate and ration (Fig. 6) a set of isopleths were constructed for net

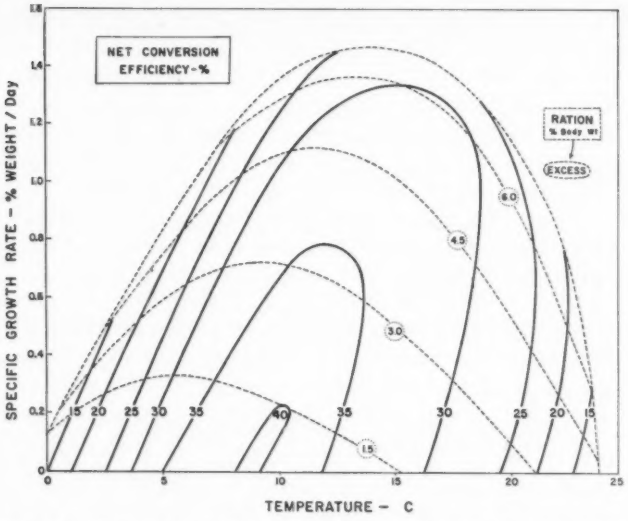

FIG. 16. Net efficiency of food conversion in relation to temperature and ration, drawn as in Fig. 14.

efficiency (Fig. 16). A maximum net efficiency, just bordering on 40%, occurred between 8 and 10 C for rations of 1.5%/day down to 0.8%/day, the maintenance level. Minimum efficiency of 15% occurred at low temperatures (1–3 C) at all accepted rations, and at 23 ± 0.5 C for rations in excess of 4%/day.

The net efficiency isopleths form a pattern opposite to those for gross efficiency, net efficiency *decreasing* with increasing growth rate (cf. Fig. 14 and 16). The "favourable axis" has a center in the same temperature region (9–10 C) but is rotated anticlockwise in a more vertical position with relation to the temperature axis. The advantage of low temperature for gross efficiency conversion is not present for net efficiency demonstrating that the process of growth, when separated from the interwoven complex of the energy requirements and food turnover associated with maintenance, has a narrower, different focal area in relation to temperature.

The combined evidence from growth rates and conversion efficiencies indicates that temperatures between 5 and 17 C provide young sockeye with the best environmental circumstance for elaborating cells and storing energy.

BODY COMPOSITION

Many factors have been shown to influence body composition (Love, 1957). In addition to quantity of food and environmental temperature, Parker and Vanstone (1966) reported that young pink salmon (*O. gorbuscha*) showed changes in composition with size, age, diet, and at particular stages of life (ontogenesis). Slight but significant changes in moisture content even occurred

diurnally. Various investigators noted that starvation is accompanied by an early decrease in fat followed by gradual protein depletion; loss of body weight is partially offset by an increase in the proportion of water (Idler and Clemens, 1959; Phillips et al., 1966).

At any one stage in life it can be expected that the balance of body constituents will be governed by the level of food intake and the rate of expenditure of energy. To a large extent energy expenditure is governed by activity and temperature. Since metabolic rate appears to be closely correlated to feeding rate (Paloheimo and Dickie, 1966a), it may be deduced that temperature will act as *a major* independent variable. It is therefore of interest to examine further some of the interrelations between food intake and temperature as they affect the body composition of young sockeye in an effort to distinguish the respective roles.

FAT PROPORTION

At all temperatures the most responsive constituent to food intake was fat, which varied from a low of 0.6% to a high of 9.5% (Table 3). If the percentage composition of fat is considered in relation to ration (Fig. 17A) there is a direct proportionality at each level of temperature, with very little distinction between the fish held at 20 C and those at 15 C. At lower temper-

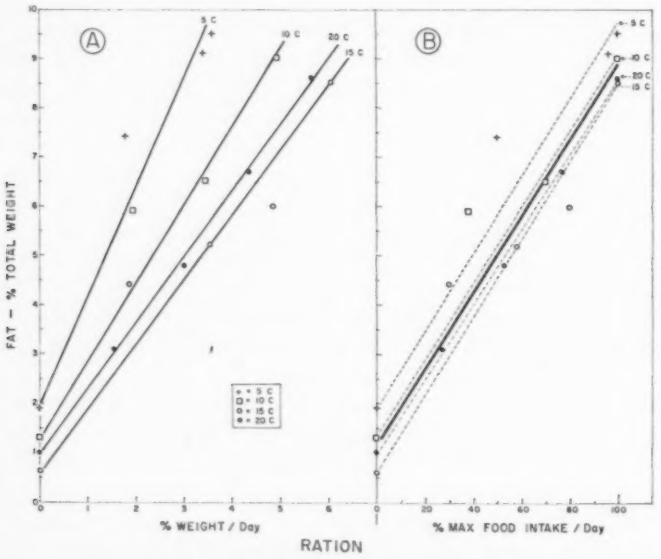

FIG. 17. Relation of body fat to daily ration expressed as a fraction of dry body weight (A) and of the maximum food intake (B), showing the relative influence of temperature. Trend lines for each temperature have been drawn by eye in A. The solid line in B has been drawn for all points, with broken lines representing the constrained effect of temperature when body fat is related to percent maximum food intake.

atures high fat fractions of about 9% occur at lower levels of daily ration, as might be expected from the reduction in maintenance associated with reduced temperature.

A refinement of the above relation may be made by expressing the ration as a fraction of the maximum daily food intake. Since the latter is exponentially temperature-dependent (see Fig. 8) a large proportion of the temperature influence is removed (Fig. 17B). Only at 5 C does there appear to be sufficient difference to warrant separate representation of the data. It is quite possible that the fish at 5 C had not reached a static balance of their body constituents at each ration, and that in time they would have been inseparable from the rest.

Aside from demonstrating the dominant role that ration plays in defining the proportion of body constituents, it is apparent that a simple determination of the fat fraction is likely to reveal at what sort of level the fish is feeding in relation to its capacity, without particular concern for temperatures. As long as some stage of metamorphosis such as smolt-transformation is not in progress the fat assessment should be of value to the ecologist as a means of assessing the general feeding level of any particular population.

Since protein tends to be the most stable component of the gross body constituents it might be thought that the fat/protein ratio (or fat to fat-free dry material) would provide a better indicator of feeding intensity. However, it will be recalled (Fig. 10) that both fat and protein respond in a similar manner to reduced ration so that with the exception of the lowest rations and highest temperatures the fat/protein ratio does not provide a useful index of the relative feeding rate.

WATER CONTENT

The starved and excess-fed fish provide examples of extremes of water content that can occur for a given species, age, and diet (71–86.9%, Table 3). Love (1957) records values for fish ranging from 53 to 89.3%, but warns that determinations are not strictly comparable because of different analytical techniques.

Despite this wide variation (which is temperature dependent on a *fixed* ration), when food is present at either the maintenance level or sufficient for maximum growth the body composition was found to be remarkably constant at all temperatures. Under maintenance conditions the water content ranged from 78.3 to 79.7%; on a maximum ration the values ranged from 72.0 to 73.0%. This demonstrates the sort of balance that is struck when particular feeding rations are considered.

CONCLUDING COMMENTS

The prime purpose was to determine how temperature affects the growth and efficiency of food conversion of young sockeye salmon at various levels of feeding intensity. The hypothesis that the optimum temperature for growth would shift to a lower temperature with a decrease in ration was supported.

A change in the optimum from 15 to 5 C occurred when the ration was reduced from 6 to 1.5%/day.

The support for the hypothesis depended on maintaining a favorable balance between reduced maintenance costs and efficiency of growth processes (including digestion and transformation) associated with lower temperatures. The fact that a gross conversion efficiency of 20% occurred at 5 C on a reduced ration lends confirmation to the conclusion that the saving in maintenance costs that low temperature affords is the main contributor to sustained efficiency. This is further resolved and supported by examining net efficiency, which indicates some interaction between temperature and food transformation in favor of sustained efficiency at reduced temperature, but not to the same extent as is the case for gross efficiency. This suggests that distinct relations exist between temperature and each of maintenance, metabolism, digestion, and transformation. Although not defined with precision, it is apparent from the high gross efficiency between 5 and 17 C that the enzymatic process of digestion is relatively temperature-independent.

A decrease in the maximum growth rate with age supports the general concept that growth rate is size-specific at any given temperature. That this is not entirely so is indicated by the duration of the growth stanzas. Thus, at 15 C on excess ration sockeye showed a relatively stable growth rate of 1.7%/day from August to January although changing in weight from 10 to 80 g. Since a variety of mathematical transformations have been applied to the growth process, it may be that use of the simple exponential equation is inadequate for a consideration of the effect of size. It is apparent from the sockeye data that the temperature optimum becomes broader with age, so that the significance of temperature to growth, in the presence of unrestricted food, is greatest in the very early stages. Additional significance may be attached to the high efficiency potential that occurs between 5 and 17 C. This temperature range characterizes much of the lacustrine distribution of the species in its early freshwater life. Experimentally, the determination of food-conversion efficiency may well offer one of the greatest sources of insight concerning what governs the success of an organism in nature.

The presence of an optimum for growth at 15 C (when fed to excess) coincides with optimum metabolic scope, greatest tolerance to oxygen-debt, and maximum sustained speed (Brett, 1964). It is apparent that a general physiological optimum occurs at this temperature in fresh water.

The potential for increasing returns in the culturing of fish is self-evident by the presence of such a wide range of efficiency. By determining the particular combination of temperature and ration that produces maximum growth a gross efficiency of 25% is predictable.

Finally, the insight gained by conducting growth × ration × temperature experiments, accompanied by determinations of body constituents, obviously offers a great deal to the general problem of interpreting ecological relations.

ACKNOWLEDGMENTS

Messrs D. B. Sutherland and G. D. Heritage have assisted by providing the careful and continuous attention necessitated by these long-term experiments. Their willing support and patience have done much to make the program possible.

As well as contributing a great deal of helpful advice, Dr T. D. D. Groves and associates conducted over half the proximate analyses. It is a pleasure to acknowledge this assistance and the cooperative interest of the University of Victoria through its Department of Biochemistry under the direction of Dr A. J. Wood.

REFERENCES

ALDERDICE, D. F., J. R. BRETT, AND D. B. SUTHERLAND. 1966. Design of small holding tank for fish. J. Fish. Res. Bd. Canada 23: 1447–1450.

AMERICAN SOCIETY FOR TESTING MATERIALS. 1966. Method D.271, for Parr bomb calorimeter. Am. Soc. Testing Materials 19: 42–46.

ANON. 1959. The Heinz handbook of nutrition. McGraw-Hill Book Co., New York. 439 p.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1960. Methods of analysis. 9th ed. Assoc. Offic. Anal. Chemists, Washington, D.C.

BALDWIN, N. S. 1957. Food consumption and growth of brook trout at different temperatures. Trans. Am. Fish. Soc. 86: 323–328.

BERTALANFFY, L. VON. 1957. Quantitative laws in metabolism and growth. Quart. Rev. Biol. 32: 217–231.

BLACK, E. C. 1958. Energy stores and metabolism in relation to muscular activity in fishes, p. 51–67. *In* P. A. Larkin [ed.] The investigation of fish-power problems. H. R. MacMillan lectures in fisheries, Univ. of British Columbia, Vancouver, B.C.

BLAXTER, K. L. [ed.] 1965. Energy metabolism. Academic Press Inc., New York. 450 p.

BRETT, J. R. 1952. Temperature tolerance in young Pacific salmon, genus *Oncorhynchus*. J. Fish. Res. Bd. Canada 9: 265–323.

1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Canada 21: 1183–1226.

1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 22: 1491–1501.

1967. Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. J. Fish. Res. Bd. Canada 24: 1731–1741.

BRODY, S. 1945. Bioenergetics and growth. Reinhold Publishing Corp., New York. 1023 p.

BROWN, M. E. 1946. The growth of brown trout (*Salmo trutta* Linn.). II. Growth of two-year-old trout at a constant temperature of 11.5°C. J. Exptl. Biol. 22: 130–144.

1957. Experimental studies of growth, p. 361–400. *In* M. E. Brown [ed.] Physiology of fishes, Vol. 1. Academic Press, Inc., New York.

DAVIES, P. M. C. 1963. Food input and energy extraction efficiency in *Carassius auratus*. Nature 4881: 707.

DAVIS, G. E., AND C. E. WARREN. 1968. Estimation of food consumption rates, p. 204–225. *In* W. E. Ricker [ed.] Methods for assessment of fish production in fresh waters. Intern. Biol. Programme Handbook 3. Blackwell Scientific Publications, Oxford and Edinburgh.

DONALDSON, L. R., AND F. J. FOSTER. 1940. Experimental study of the effect of various water temperatures on the growth, food utilization, and mortality rates of fingerling sockeye salmon. Trans. Am. Fish. Soc. 70: 339–346.

FISHER, K. C. 1958. An approach to the organ and cellular physiology of adaptation to temperature in fish and small mammals, p. 3–39. *In* C. L. Prosser [ed.] Physiological adaptation. Am. Physiol. Soc., Washington, D.C.

FOLCH, J., M. LEE, AND G. H. S. STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509.

FRY, F. E. J. 1947. Effects of the environment on animal activity. Univ. Toronto Studies, Biol. Ser. 55, Publ. Ont. Fish. Res. Lab. 68: 1–62.

GERKING, S. D. 1962. Production and food utilization in a population of bluegill sunfish. Ecol. Monographs 32: 31–78.

HALVER, J. E., AND W. E. SHANKS. 1960. Nutrition of salmonoid fishes. VIII. Indispensable amino acids for sockeye salmon. J. Nutr. 72: 340–346.

HASKELL, D. C., L. E. WOLF, AND L. BOUCHARD. 1956. The effect of temperature on the growth of brook trout. New York Fish Game J. 3: 108–113.

HATANAKA, A., AND MASAO TAKAHASHI. 1960. Studies on the amounts of the anchovy consumed by the mackerel. Tohoku J. Agr. Res. 11: 83–100.

HOAR, W. S. 1966. General and comparative physiology. Prentice-Hall, Inc., New Jersey. 815 p.

HOCHACHKA, P. W., AND A. C. SINCLAIR. 1962. Glycogen stores in trout tissues before and after stream planting. J. Fish. Res. Bd. Canada 19: 127–136.

HUBBS, C. L., AND C. HUBBS. 1953. An improved graphical analysis and comparison of series of samples. Systematic Zool. 2: 49–56.

IDLER, D. R., AND W. A. CLEMENS. 1959. The energy expenditures of Fraser River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. Intern. Pacific Salmon Fish. Comm. Progr. Rept. 80 p.

IVLEV, V. S. 1945. [The biological productivity of waters.] Usp. Sovrem. Biol. 19: 98–100. (In Russian; Transl. in J. Fish. Res. Bd. Canada 23: 1727–1759.)

JENNINGS, J. B. 1965. Feeding, digestion and assimilation in animals. Pergamon Press, London and New York. 228 p.

JOHNSON, W. E. 1965. On mechanisms of self-regulation of population abundance in *Oncorhynchus nerka*. Mitt. Intern. Ver. Limnol. 13: 66–87.

KEVERN, N. R. 1966. Feeding rate of carp estimated by a radioisotopic method. Trans. Am. Fish. Soc. 95: 363–371.

KINNE, O. 1960. Growth, food intake, and food conversion in a euryplastic fish exposed to different temperatures and salinities. Physiol. Zool. 33: 288–317.

KLEIBER, M. 1961. The fire of life. An introduction to animal energetics. John Wiley & Sons, Inc., New York. 454 p.

LOVE, R. M. 1957. The biochemical composition of fish, p. 401–418. *In* M. E. Brown [ed.] Physiology of fishes, Vol. 1. Academic Press, Inc., New York.

MAGNUSON, J. J. 1962. An analysis of aggressive behavior, growth and competition for food and space in medaka, *Oryzias latipes* (Pisces, Cyprinodontidae). Canadian J. Zool. 40: 313–363.

NARVER, D. W. MS, 1967. Diel vertical movement of pelagial sockeye salmon juveniles. Fish. Res. Bd. Canada, MS Rept. Ser. 949: 24–28.

NEEDHAM, A. E. 1964. The growth process in animals. Sir Isaac Pitnam and Sons Ltd., London. 522 p.

PALOHEIMO, J. E., AND L. M. DICKIE. 1966a. Food and growth of fishes. II. Effects of food and temperatures on the relation between metabolism and body weight. J. Fish. Res. Bd. Canada 23: 869–908.

1966b. Food and growth of fishes. III. Relations among food, body size, and growth efficiency. J. Fish. Res. Bd. Canada 23: 1209–1248.

PANDIAN, T. J. 1967a. Intake, digestion, absorption and conversion of food in the fishes *Megalops cyprinoides* and *Ophiocephalus striatus*. Marine Biol. 1: 16–32.

1967b. Transformation of food in the fish *Megalops cyprinoides*. I. Influence of quality of food. Marine Biol. 1: 60–64.

PARKER, R., AND W. E. VANSTONE. 1966. Changes in chemical composition of central British Columbia pink salmon during early sea life. J. Fish. Res. Bd. Canada 23: 1353–1384.

PENTELOW, F. T. K. 1939. The relation between growth and food consumption in the brown trout (*Salmo trutta*). J. Exptl. Biol. 16: 446–473.

PHILLIPS, A. M., JR., D. L. LIVINGSTON, AND H. A. POSTON. 1966. The effect of changes in protein quality, calorie sources and calorie levels upon the growth and chemical composition of brook trout. N. Y. State Dept. Conserv. Fish. Res. Bull. 29: 6–7.

SMIT, H. 1967. Influence of temperature on the rate of gastric juice secretion in the brown bullhead (*Ictalurus nebulosus*). Comp. Biochem. Physiol. 21: 125–132.

STRAWN, K. 1961. Growth of largemouth bass at various temperatures. Trans. Am. Fish. Soc. 90: 334–335.

SWIFT, D. R. 1955. Seasonal variations in the growth rate, thyroid gland activity and food reserves of brown trout (*Salmo trutta* Linn.). J. Exptl. Biol. 32: 751–764.

1964. The effect of temperature and oxygen on the growth rate of the Windermere char, *Salvelinus alpinus* (Willughbii). Comp. Biochem. Physiol. 12: 179–183.

TAYLOR, C. 1962. Growth equations with metabolic parameters. J. Conseil, Conseil Perm. Intern. Exploration Mer 27: 270–286.

THOMPSON, D. H. 1941. The fish production of inland streams and lakes. Symp. Hydrobiol., Univ. Wisconsin Press, Madison, Wis. p. 206–217.

VINOGRADOV, A. P. 1953. The elementary chemical composition of marine organisms. Efron and Setlow [translators]. Yale Univ. Press, New Haven, Conn. p. 463–566.

WARREN, C. E., AND G. E. DAVIS. 1967. Laboratory studies on the feeding, bioenergetics and growth of fish, p. 175–214. *In* S. D. Gerking [ed.] The biological basis for freshwater fish production. Blackwell Scientific Publications, Oxford.

WEST, B. W. 1966. Growth rates at various temperatures of the orange-throat darter *Etheostoma spectabilis*. Arkansas Acad. Sci. Proc. 20: 50–53.

WINBERG, G. G. 1956. Rate of metabolism and food requirements of fishes. Belorussian State Univ., Minsk. 251 p. (Fish. Res. Bd. Canada Transl. Ser. 194.)

(Appendices A–B *follow*)

APPENDIX A.

INGREDIENTS AND PERCENTAGE COMPOSITION BY WEIGHT OF DIET USED IN SOCKEYE GROWTH EXPERIMENTS, 1964–67.

	%	
Water	37.2	
Drained, canned salmon	29.7	
Clark's pellet feed	11.1	(J. R. Clark & Co., Salt Lake City, Utah)
Fresh beef liver	10.0	
Yeast	3.7	
Pablum mixed cereal	2.2	(Edward Dalton Co., Toronto, Ont.)
Cod-liver oil	2.2	
Vitamin pack	1.5	(Nutritional Biochemicals Corp., Cleveland, Ohio)
Gelatin	1.5	
Iodized salt	0.9	

APPENDIX B.

PROCEDURE FOR DRY EXTRACTION OF FAT[6]

Frozen fish were sliced longitudinally and their abdominal cavities cut open. The fish were then placed on tared aluminum foil trays and dried to constant weight in a forced air oven at 80 C. The fish were not left in the oven any longer than necessary in order to minimize fat oxidation.

The dried fish were crushed and transferred, along with the aluminum foil, to a 500-ml Erlenmeyer fitted with an aluminum foil-wrapped stopper. The material in the flask was extracted with 200 ml of *n*-hexane. The foil was then removed and the sample was further extracted with three successive 100-ml portions of hexane. Each extraction proceeded with occasional shaking for 24 hr. After each extraction the hexane was poured off through a small tared filter paper to prevent loss of suspended particulate material. Final traces of hexane were removed from the fish in a vacuum oven at 40 C.

Subsequent ash (predominantly as oxides) and total protein determinations carried out on the fat-extracted material accounted for 99.5% of the sample.

[6]Method used by Dr T. D. D. Groves, Department of Biochemistry, University of Victoria, Victoria, B.C.

Patterns of Insecticide Resistance in the Mosquitofish, *Gambusia affinis*[1]

DUDLEY D. CULLEY, JR.[2] AND DENZEL E. FERGUSON

Department of Zoology, Mississippi State University
State College, Mississippi 39762, USA

CULLEY, DUDLEY D., JR., AND DENZEL E. FERGUSON. 1969. Patterns of insecticide resistance in the mosquitofish, *Gambusia affinis*. J. Fish. Res. Bd. Canada 26: 2395–2401.

The extent of insecticide resistance in a resistant population of mosquitofish (*Gambusia affinis*) from Belzoni, Mississippi, was compared with that of a susceptible population from State College, Mississippi, using 28 insecticides of five major groups. Results of 48-hr bioassays show that resistant mosquitofish have developed high resistance only to the toxaphene–endrin related insecticides, even though insecticides from other groups were heavily applied. Spray records for the Belzoni area and insecticide characteristics such as stability and toxicity aided in evaluating cross-resistance patterns in the resistant population. Patterns of resistance in mosquitofish are similar to those in many resistant arthropods.

Received March 11, 1969

INTRODUCTION

RESISTANCE TO INSECTICIDES has been well established for field populations of fishes (Vinson et al., 1963; Boyd and Ferguson, 1964a; Ferguson et al., 1964, 1965; Ferguson and Bingham, 1966). To date six species of fish collected in the agricultural region of Mississippi are known to be resistant to over 20 insecticides, with tolerances ranging from 2 to 1500×. Cross resistance is suspected since fish are resistant to some insecticides that have not been applied in the areas (Ferguson et al., 1966; Boyd and Ferguson, 1964b).

Our study was designed to determine the extent of resistance and to identify the patterns of resistance in a field population of mosquitofish having a history of exposure to various insecticides.

METHODS

Resistant mosquitofish from a drainage canal near Belzoni, Mississippi, and a susceptible population from near State College, Mississippi, an area with no history of insecticide application, were used to bioassay 28 insecticides representing five major groups of chlorinated hydrocarbon and organophosphate insecticides (Table 1).

The fish were treated 24 hr in 2.5‰ noniodized table salt to rid them of fungus and tail rot. Then the fish were put in fresh water, and held 1–5 days before testing. LC_{50} values for salt

[1]Supported by grant ES 00086 from the U.S. Public Health Service and contract 12-14-100-6895 (33) from the Agricultural Research Service, USDA.

[2]Present address: Louisiana State University, Baton Rouge, La.

Printed in Canada (J1406)

TABLE 1. Major groups (Kenaga, 1966) and purity of insecticides used in 48-hr bioassay tests of resistant and susceptible populations of mosquitofish.

Chlorinated hydrocarbons	
Group I. Diphenyl aliphatics	
DDT	100%
Perthane	95%
Methoxychlor	Technical
Kelthane	"
Dimite	"
Group II. Chlorinated aryl hydrocarbons	
Toxaphene	100%
Strobane	100%
Lindane	Technical
Chlordane	100%
Heptachlor	74%
Dieldrin	95%
Aldrin	95%
Endrin	97%
Organophosphates	
Group III. Aliphatic derivatives	
Ethion	95%
TEPP	Technical
Phosdrin	100%
Malathion	95%
Phosphamidon	80%
Azodrin	Technical
Bidrin	90%
OMPA	90%
Group IV. Phenyl derivatives	
Parathion	Technical
Methyl Parathion	Technical
Methyl Trithion	91%
Ronnel	Technical
Group V. Heterocyclic derivatives	
Dursban	Technical
Guthion	90%
CO-RAL	Technical

for several species of fish are much higher than concentrations used here (McKee and Wolf, 1963). Testing began when fungus and tail rot were controlled. Although the fish were fed a synthetic diet daily during the holding period, no food was given 24 hr before testing. Water temperature in the holding vats was adjusted to within 5 F of the test solution temperature.

Comparison of salt-treated fish with those not treated in salt using seven insecticides indicated no difference in mortality in 48-hr tests. However, populations held for testing and not treated with salt often developed advanced cases of tail rot, and many died during the 5-day holding period. Prevalence of tail rot in the population, difficulty of detection in early stages, and the large numbers of fish needed for this study made the control of tail rot mandatory.

Static bioassay techniques — One percent stock solutions of insecticide dissolved in acetone were prepared monthly and refrigerated at about 35 F. Insecticide solutions were brought to room temperature (72±3 F) for pipetting. The stock solution was pipetted into a 10-liter

mixing jar containing 3–4 ml of acetone per liter of test solution to be mixed. This was diluted with tap water (72±3 F, hardness as $CaCO_3$ 28 ppm, pH 7.8) to prepare the final test concentrations. Previous tests showed that mosquitofish tolerated a 36-hr exposure to 1% acetone in water. The solution was mixed thoroughly and poured into test containers. Controls were placed in tap water and acetone.

The approximate 48-hr LC_{50} value was determined in preliminary tests with 2.5 liters of insecticide solution in 1-gal jars. Two jars of 6 fish each (ca. 0.5 g fish/liter) were tested on three or four concentrations of the insecticide. Fish were put in the solutions within an hour because preliminary testing suggested that the LC_{50} values increased if the prepared solutions were allowed to stand several hours.

When preliminary tests with susceptible mosquitofish showed that an insecticide had an LC_{50} value above 3500 ppb, the compound was excluded. In the case of other insecticides, further tests were run in 15-gal aquaria using a restricted range of concentrations. The procedures used in preliminary tests were repeated except that 50 fish and 20 liters of test solution were used. The LC_{50} value for each insecticide tested was confirmed in a single aquarium using that concentration.

Fish less than 25 mm in total length are more susceptible to injury and were excluded from the tests. Any fish that died or exhibited unusual symptoms within 15 min after being placed in test solutions were removed, but not replaced. Several insecticides produced shock reactions for several minutes when fish were placed in the solution, but no deaths occurred, and such fish were not removed. All mortality was recorded at 12-hr intervals, and dead fish were removed.

Confidence intervals (95%) were determined for the 48-hr LC_{50} values (ppb) by probit analysis, a statistical procedure outlined by Daum et al. (1965) for use on the IBM 360 and 1620 digital computers. Data from a minimum of three concentrations were necessary for calculating the LC_{50} values, providing variability in mortality was small at each concentration; four concentrations were used in nearly all tests.

Test containers were cleaned by scrubbing them with an abrasive soap, after which clean water was run through them for 2 days. Between tests, cleanliness of testing apparatus was monitored by bioassaying susceptible fish in 1–2 inches of water in cleaned containers. If no mortality or signs of stress occurred in 48 hr the containers were considered to be clean.

A list of insecticides applied in the Belzoni study area between 1964 and 1967 was obtained from landowners with land that drained into the Belzoni canal (Table 2). These records and toxicity data for the susceptible mosquitofish aided in identifying insecticides most likely responsible for the resistance and cross-resistance reported.

TABLE 2. Insecticides applied in the Belzoni study area for 1964–67.

Insecticide	Year			
	1964	1965	1966	1967
Bidrin	**[a]			
DDT			*[b]	*
Endrin	*	*	**	
Guthion		**		
Lindane			**	
Malathion	**	**		
Phosphamidon		**		
Methyl Parathion	*	*	*	*
Sevin			**	
Toxaphene			**	*

[a] ** = minor use.
[b] * = used in 80–90% of all applications by all landowners.

RESULTS

High levels of resistance in the Belzoni population have developed only to the group II insecticides (Table 3). Two levels of resistance are apparent — a high level of at least 357× includes endrin, Strobane, heptachlor, and toxaphene — a lower level (less than 100×) includes chlordane, aldrin, dieldrin, and lindane. All differences between the resistant and susceptible populations are significant (.05 level).

Little resistance has developed to insecticides in the other four groups. The highest resistance levels outside the group II insecticides were to DDT (5.1×), a group I insecticide, ethion (4.8×), a group III insecticide, and parathion (4.1×), a group V compound.

Six insecticides (phosphamidon, Azodrin, Bidrin, OMPA, methyl parathion, and CO-RAL) were excluded from further testing when the 48-hr LC_{50} for the susceptible population exceeded 3500 ppb.

DISCUSSION AND CONCLUSIONS

Two main points stand out in this study. First, high resistance has developed to the group II insecticides, yet only two of these compounds (toxaphene and endrin) were used during the past 4 years. Second, little resistance has developed to other insecticides even though many of them have been used for insect control in the area (Table 2). For example, methyl parathion (group IV) has been used extensively along with lesser amounts of malathion and phosphamidon (group III), and Guthion (group V). DDT (group I), a chlorinated hydrocarbon, has been used in large quantities for the past 2 years.

The use patterns and the high toxicity of DDT, toxaphene, and endrin most likely provided enough selective pressure for resistance to develop to DDT (group I) and the group II insecticides. The lesser levels of resistance to group I insecticides, other than DDT, and to the insecticides in groups III, IV, and V, could be cross tolerances produced by DDT, toxaphene, endrin selection, or all three. Unpublished data from laboratory selection studies by the authors support this explanation.

The combined selective action of several organophosphate insecticides may have accounted for the tolerances observed. However, the low toxicity of methyl parathion (Table 3), and the small amount of Guthion, malathion, and phosphamidon used in the Belzoni study area probably have not generated sufficient selective pressure to produce resistance. Gas chromatographic analyses reveal only trace quantities of these insecticides in the Belzoni canal (J. L. Ludke, personal communication). Selection with parathion over the past 4 years in the laboratory has failed to produce tolerances comparable with that in the Belzoni fish.

The resistance pattern in the Belzoni population will probably never be completely understood because study of the population began after resistance had already developed. Laboratory selection studies presently underway

TABLE 3. Comparative 48-hr LC_{50} values (ppb) for resistant (Belzoni) and susceptible (State College) populations of mosquitofish tested on 28 insecticides representing five major insecticide groups.

Insecticide	LC_{50} Susceptible (State College)	LC_{50} Resistant (Belzoni)	Fold difference
Chlorinated hydrocarbons			
Group I			
DDT	19	96	5.1[a]
Dimite	3,094	3,872	1.3[a]
Methoxychlor	109	186	1.7[a]
Perthane	479	1,542	3.2[a]
Kelthane	777	2,121	2.7[a]
Group II			
Endrin	0.6	314	523.3[a]
Strobane	11	6,253	568.4[a]
Heptachlor	35	12,500	357.1[a]
Toxaphene	12	4,519	376.5[a]
Chlordane	41	3,804	92.8[a]
Aldrin	36	2,558	71.1[a]
Dieldrin	8	434	54.3[a]
Lindane	74	3,104	41.9[a]
Organophosphates			
Group III			
Ethion	206	983	4.8[a]
TEPP	2,845	3,500	1.2[a]
Phosdrin	2,914	2,935	1.0[a]
Malathion	1,061	841	.8
Phosphamidon	10,000[b]	–	–
Azodrin	10,000[b]	–	–
Bidrin	10,000[b]	–	–
OMPA	10,000[b]	–	–
Group IV			
Parathion	48	199	4.1[a]
Methyl Trithion	720	1,078	1.5[a]
Ronnel	1,450	1,753	1.2[a]
Methyl Parathion	4,000	–	–
Group V			
Dursban	1,018	1,291	1.3[a]
Guthion	68	79	1.2
CO-RAL	3,500	–	–

[a]Resistant strain significantly higher (.05 level).
[b]Discontinued because the State College population LC_{50} exceeded 3,500 ppb.

should provide further insight into the development of patterns of resistance and cross-resistance in field populations of fishes.

Species of fish in other areas of the Mississippi Delta have been exposed to various combinations of insecticides and have developed resistance patterns similar to the Belzoni mosquitofish. For example, mosquitofish from near Sidon, Mississippi, showed high resistance (11–300×) to the group II insecticides endrin, aldrin, dieldrin, toxaphene, heptachlor, lindane, and Strobane (Boyd and Ferguson, 1964a). Resistance to the group I insecticides dilan, methoxychlor, thanite, Kelthane, Perthane, and DDT ranged from .67- to 4.1-fold in the Sidon population (Boyd and Ferguson, 1964b). No resistance was found to the organophosphates methyl parathion, malathion, TEPP, Delnav, Ronnel, and Methyl Trithion, which represent groups III, IV, and V, but resistance to parathion (erroneously labelled methyl parathion in the original paper) was noted (Ferguson and Boyd, 1964).

Bluegills (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), and golden shiners (*Notemigonus crysoleucas*) from near Indianola, Mississippi, have 36–200× resistances to the group II insecticides toxaphene, aldrin, dieldrin and endrin, but no resistance to DDT, a group I insecticide, even though it had been applied in the area (Ferguson et al., 1964).

High endrin resistance (group II) has been reported for the yellow bullhead (*Ictalurus natalis*) by Ferguson and Bingham (1966), but no data are available for this species on other insecticides.

Comparison of resistance patterns in arthropods and resistant fishes reveals both similarities and differences. The high resistance to group II insecticides observed in fish is apparently common in field populations of arthropods (Brown, 1958). Little resistance to the organophosphates has been found in resistant fishes, and this is also true of many arthropods, even those selected with an organophosphate (Brown and Adebi, 1960; Heuval and Cochran, 1965). Although high DDT resistance is common in insect populations (Brown, 1958), little DDT resistance has developed in the Belzoni or other populations of resistant fishes (Ferguson et al., 1964, 1965).

The widespread use of DDT, its stability, and the absence of DDT-resistant fish populations emphasize the danger of DDT, even though it is not the most toxic insecticide. The high toxicity and stability of insecticides such as endrin, the high levels of resistance that have developed from exposure to such compounds, and the ability of resistant fish to concentrate massive body burdens of residues pose a potential problem (Rosato and Ferguson, 1968). A long-term diet of field-collected mosquitofish from heavily contaminated sites will kill susceptible predaceous fishes (Ferguson et al., 1967).

REFERENCES

BOYD, C. E., AND D. E. FERGUSON. 1964a. Susceptibility and resistance of mosquitofish to several insecticides. J. Econ. Entomol. 57: 430–431.

1964b. Spectrum of cross-resistance to insecticides in the mosquitofish (*Gambusia affinis*). Mosquito News 24: 19–21.

BROWN, A. W. A. 1958. Insecticide resistance in arthropods. Monograph Ser. 38, World Health Organ., Geneva. 240 p.

BROWN, A. W. A., AND Z. H. ABEDI. 1960. Cross resistance characteristics of a malathion-tolerant strain developed in *Aedes aegypti*. Mosquito News 20: 118–124.

DAUM, R. J., C. GIVENS, AND W. KILLCREAS. 1965. Probit analysis, Fortran 360 E-level. Computing Center, Mississippi State Univ., State College, Mississippi. 17 p.

FERGUSON, D. E., AND C. R. BINGHAM. 1966. Endrin resistance in the yellow bullhead (*Ictalurus natalis*). Trans. Am. Fish. Soc. 95: 325–326.

FERGUSON, D. E., AND C. E. BOYD. 1964. Apparent resistance to methyl parathion in mosquitofish (*Gambusia affinis*). Copeia 1964: 706.

FERGUSON, D. E., W. D. COTTON, D. T. GARDNER, AND D. D. CULLEY. 1965. Tolerances to five chlorinated hydrocarbon insecticides in two species of fish from a transect of the lower Mississippi River. J. Miss. Acad. Sci. 11: 239–245.

FERGUSON, D. E., D. D. CULLEY, W. D. COTTON, AND R. P. DODDS. 1964. Resistance to chlorinated hydrocarbon insecticides in three species of freshwater fish. BioScience 14: 43–44.

FERGUSON, D. E., D. T. GARDNER, AND A. L. LINDLEY. 1966. Toxicity of Dursban to three species of fish. Mosquito News 26: 80–82.

FERGUSON, D. E., J. L. LUDKE, M. T. FINLEY, AND G. G. MURPHY. 1967. Insecticide-resistant fishes: a potential hazard to consumers. J. Miss. Acad. Sci. 13: 138–140.

HEUVAL, M. J., AND D. G. COCHRAN. 1965. Cross resistance to organophosphorus compounds in malathion and Diazinon resistant strains of *Blattella germanica*. J. Econ. Entomol. 58: 872–874.

KENAGA, E. E. 1966. Commercial and experimental organic insecticides (1966 revision). Bull. Entomol. Soc. Am. 12: 161–217.

MCKEE, J. E., AND H. W. WOLF. 1963. Water quality criteria. State Water Quality Control Board Publ. 3-A, Sacramento, California. 548 p.

ROSATO, P., AND D. E. FERGUSON. 1968. The toxicity of endrin-resistant mosquitofish to eleven species of vertebrates. BioScience 18: 783–784.

VINSON, S. B., C. E. BOYD, AND D. E. FERGUSON. 1963. Resistance to DDT in the mosquitofish (*Gambusia affinis*). Science 139: 217–218.

The Zoarcid *Lycodopsis pacifica* in Outer Burrard Inlet, British Columbia[1]

C. D. LEVINGS[2]

Institute of Fisheries and Department of Zoology
University of British Columbia, Vancouver 8, B.C.

LEVINGS, C. D. 1969. The zoarcid *Lycodopsis pacifica* in outer Burrard Inlet, British Columbia. J. Fish. Res. Bd. Canada 26: 2403–2412.

Aspects of the biology of *Lycodopsis pacifica* were investigated during December 1965–November 1966 in outer Burrard Inlet, British Columbia. Trawls were used as sampling devices.

After approximately age III, males appeared to have a different growth pattern than females. Females apparently had higher mortality rates than males. Spawning occurred from late August to January. Most males were mature at a length of 170 mm, and most females at 160 mm. The complements of mature eggs were small (average approximately 30). The mature eggs are large, with an average diameter of 5.0 mm. There is some evidence that parental care is involved in reproductive behaviour. The species fed primarily on molluscs, annelids, and Crustacea in the bottom mud.

Received August 19, 1968

INTRODUCTION

THE PRESENT PAPER reports the results of a general biological investigation of *Lycodopsis pacifica* (Collett) 1879 captured in outer Burrard Inlet, British Columbia. Little is known of the biology of zoarcid fishes found in coastal British Columbia waters. *Lycodopsis pacifica* and commercial pink shrimp (*Pandalus borealis*) appear to have similar substrate preferences, since *L. pacifica* is often captured in large numbers in commercial shrimp trawls (Butler, MS, 1949). Field experiments related catches of *L. pacifica* to sediment type at outer Burrard Inlet and another shrimp ground in the Strait of Georgia (Levings, MS, 1967).

The taxonomy and distribution of *L. pacifica* have been described by Bayliff (MS, 1954). The species ranges from southern California to Afognak Island, Alaska, and possibly further west to Unalaska Island. It has been captured over a wide range of depths (5 fath (9.2 m) in Puget Sound; 218 fath (392 m) in California waters). Brief observations on the biology of *L. pacifica* are given in Clemens and Wilby (1961).

---

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Zoology, University of British Columbia, Vancouver, B.C.

[2]Present address: Institute of Oceanography, Dalhousie University, Halifax, N.S.

Printed in Canada (J1180)

MATERIALS AND METHODS

Outer Burrard Inlet is the seaward (western) enlargement of a coastal fjord, Burrard Inlet, on the southern British Columbia coast (Fig. 1). This area is bordered on the north by Howe Sound, on the west by the Strait of Georgia, and on the southwest by the northern arm of the Fraser River. Hydrographic data of outer Burrard Inlet have recently been reported by Waldichuk et al. (MS, 1968).

FIG. 1. Fishing areas and bathymetry of outer Burrard Inlet (lat 49°18′, long 123°11′). Depths in fathoms.

Seventeen samples were obtained from outer Burrard Inlet over the period December 1965–November 1966 (Table 1), 14 in cooperation with commercial shrimp fishermen, and 3 in experimental sampling. The number of fish in each sample ranged from 68 to 376.

Net dimensions and mesh sizes were variable (Table 2). Commercial shrimp fishermen used modified beam trawls, but otter trawls were used in all other sampling.

After capture, specimens of *L. pacifica* were fixed in a solution of 10% formalin for approximately 24 hr. They were then immersed in running fresh water for 3–5 hr, and preserved in 40% isopropyl alcohol.

All fish were measured (Fig. 2), and individuals for laboratory study were obtained nonselectively, but not necessarily randomly, from the specimens in the samples. Length measurements were taken from the tip of the snout (mouth closed) to the end of the caudal fin, to the nearest millimeter. Except where noted, all measurements were made on preserved specimens. One hundred and eight fresh specimens for a study of sexual dimorphism were obtained through the courtesy of the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C., at another area in the Strait of Georgia (Cape Lazo gully; lat 49°37′, long 124°48′) on March 18, 1967.

For each of the 17 samples, the otoliths of 20 fish were examined. The age of each was estimated using the left sacculus otolith, illuminated with reflected light. Translucent zones were counted with a binocular microscope of ×10 magnification. All otoliths were read once, then re-read a few days later without reference to length or sex of the fish or to previous readings. If the two age estimates differed, a third reading was made. The presence of a translucent zone on the outer perimeter of each otolith was noted. Irie (1960) showed experimentally that the translucent zones of otoliths, representing periods of reduced growth, may be caused by temperature-dependent phenomena. To determine the times of deposition of these zones, it is necessary to observe the otoliths over at least a 1-year period.

TABLE 1. Dates, areas, and sources for samples from outer Burrard Inlet.

Date	Sample no.	Area of fishing
Dec. 3, 1965	1	West Vancouver
Jan. 9, 1966	2	West Vancouver
Jan. 31, 1966	3	Fraser Delta
Feb. 14, 1966	4	Jericho
Mar. 6, 1966	5	West Vancouver
Mar. 21, 1966	6	West Vancouver
Apr. 10, 1966	7	Gibsons
May 4, 1966	8	West Vancouver
June 9, 1966	9[a]	Spanish Banks
July 4, 1966	10	West Vancouver
July 21, 1966	11	West Vancouver
Aug. 8, 1966	12[a]	Spanish Banks
Aug. 21, 1966	13	West Vancouver
Sept. 8, 1966	14[a]	Spanish Banks
Sept. 24, 1966	15	West Vancouver
Oct. 12, 1966	16	" "
Nov. 13, 1966	17	" "

[a]Sample obtained in experimental trawling (as on p. 2404); all others from commercial shrimp vessels.

TABLE 2. Pertinent dimensions of trawls employed at the two study areas.

Area and vessels employed	Length of foot rope ft (*m*)	Mesh size (*inches and cm*)		
		Body and wing	Codend	Liner
Cape Lazo gully:				
"Investigator No. 1"	47.3 (14.2)	1.5 (3.8)	1.5 (3.8)	0.5 (1.3)
Outer Burrard Inlet:				
Commercial shrimp vessels	43.0 (12.9)	1.3 (3.2)	1.3 (3.2)	None
Institute of Fisheries vessel	12.0 (3.6)	1.0 (2.5)	1.0 (2.5)	0.5 (1.3)

The age estimates were categorized by sex and length of the fish, then subsampled at a level of 10 fish per 5-mm length group. Because of mesh selection, the lower age and length groups were not well represented in samples from the commercial shrimp vessel catches. Supplemental data were obtained from specimens taken in experimental sampling in May and June 1966 (Levings, MS, 1967). A total of 236 (129 male and 107 female) age estimations were utilized under the subsampling scheme (Table 3). Estimates of total mortality rates were obtained from age distributions of fish captured by commercial shrimp vessels only.

The contents of the intestinal tracts were determined, and all identifiable items enumerated. Annelid worms were often broken, and only sections 1 cm long or greater were counted as an item.

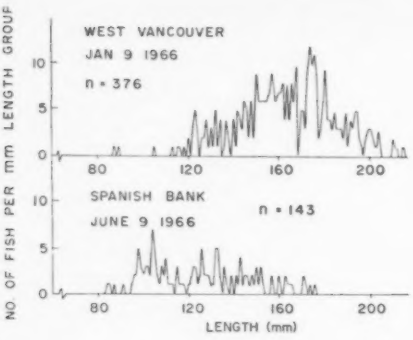

FIG. 2. Size composition of two catches of *L. pacifica* at outer Burrard Inlet. See Table 2 for dimensions of sampling gear.

TABLE 3. Age–length distributions for male and female *L. pacifica* collected at the outer Burrard Inlet study area. Data obtained by subsampling (see p. 2405).

Length (*cm*)	Male					Female				
	I	II	III	IV	V	I	II	III	IV	V
7	4	–	–	–	–	3	–	–	–	–
8	13	–	–	–	–	11	–	–	–	–
9	4	–	–	–	–	5	–	–	–	–
10	1	1	–	–	–	2	–	–	–	–
11	5	4	–	–	–	3	3	–	–	–
12	3	6	1	–	–	7	7	–	–	–
13	1	4	2	–	–	2	7	1	–	–
14	–	9	0	–	–	–	7	4	–	–
15	–	2	8	–	1	–	1	9	–	–
16	–	2	7	3	0	–	–	4	4	1
17	–	–	3	6	0	–	–	6	5	2
18	–	–	3	5	2	–	–	1	6	–
19	–	–	1	6	1	–	–	2	3	–
20	–	–	2	4	3	–	–	–	–	–
21	–	–	–	4	2	–	–	–	–	–
22	–	–	–	0	4	–	–	–	–	–
23	–	–	–	0	–	–	–	–	–	–
24	–	–	–	2	–	–	–	–	–	–
Total	31	28	27	30	13	33	25	27	18	3
Mean length	9.1	13.1	16.1	18.8	20.0	9.6	12.3	15.8	17.4	17.6
SD	1.78	1.55	1.94	2.09	2.08	1.90	2.78	1.54	1.05	0.35

An additional 10 fish were chosen for an examination of the gonads, so that 30 fish from each sample were examined for reproductive condition. Testes dimensions and egg diameters were determined with dividers to the nearest millimeter. For each fish the length and width of one testis lobe was measured, and its surface area calculated. Three egg size classes were recognized, as follows: small — approximately 0.2–1 mm diam; medium — 1–2 mm; and large — greater than 2 mm. The numbers of each size class were counted.

AGE AND GROWTH

Translucent zones on the outer perimeter of the otoliths occurred most frequently in winter months when the environmental temperature of *L. pacifica* was lowest (Fig. 3). This suggests the translucent zones are annual phenomena.

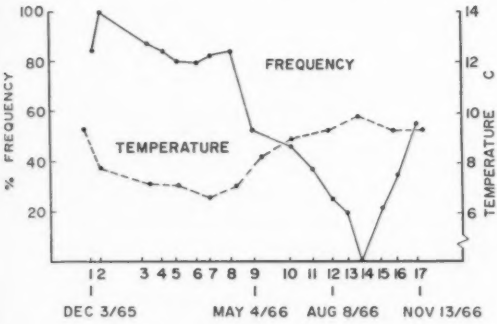

FIG. 3. Frequency distribution of *L. pacifica* otoliths with a translucent outer perimeter during the period December 1965–November 1966. Data from 20 fish for each of the samples taken on dates shown in Table 1. Temperature data from Hollister (MS, 1960).

Both males and females ranged in age from I to V years (Table 3).

Mean lengths at various ages (Fig. 4) suggest that from approximately age III to age V (170 mm or more in length) males are somewhat longer than females. Regression lines expressing growth over the period age III–V for the two sexes were as follows:

$$\text{Males:} \quad Y = 101.519 + 20.607X$$
$$\text{Females:} \quad Y = 119.077 + 12.972X$$

where Y = length (millimeters) and X = age (years).

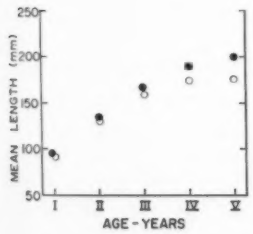

FIG. 4. Mean length-at-age relationships for the two sexes of *L. pacifica*. Data for 129 males (●) and 107 females (○). See also Table 3.

A *t*-test for differences between the slopes of the two equations (Steel and Torrie, 1960) yielded a value of 4.22, significant at $P = 0.05$. Males apparently maintain a faster growth rate than females throughout their life, or they may begin a separate "growth stanza" (Martin, 1949) at approximately age III.

There is strong sexual dimorphism in *L. pacifica* (Bayliff, MS, 1954). The sexual dimorphism is exemplified in head size of males, particularly snout length. It was not known whether this dimorphism was present at all lengths (and ages).

Snout length was estimated by the length of the maxillary (Gregory, 1933). The ratio of maxillary length to total length was the same (ca. 0.080) for males and females up to about 170 mm in length. No females larger than 170 mm were encountered. Beyond 170 mm, the ratio in males increased to ca. 0.120 (Fig. 5). This length closely coincides with length at maturity for

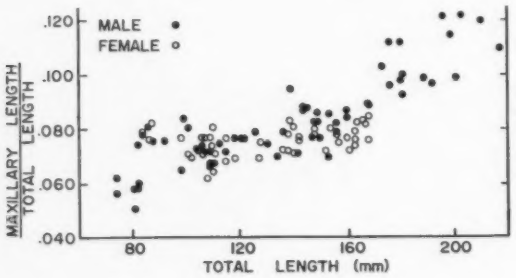

FIG. 5. Ratios of maxillary to total length for males and females of *L. pacifica*. Data for 108 fresh specimens (62 males and 46 females) collected at the Cape Lazo gully on March 18, 1967.

males (p. 2407) and with the length at which the male growth pattern diverged from that of females (Fig. 4).

MORTALITY ESTIMATES

The numbers of males and females in various age-groups in the 14 samples from commercial shrimp vessels were:

Age (*years*):	I	II	III	IV	V
Males	3	16	37	46	18
Females	1	23	51	31	4

Using the method of Jackson (1939, cited in Ricker, 1958), weighted estimates of annual mortality rates were obtained. Only fish age III and older were considered. Males had a mortality rate of 0.229 whereas females had a rate of 0.574. Over the age span considered, females apparently had a higher total annual mortality rate.

REPRODUCTION AND PARENTAL CARE

Males — The bilobed testes of *L. pacifica* lie in the posterodorsal part of the body cavity. Each lobe of the testis is roughly rectangular in shape. The testes are light grey in colour; their whiteness seems to accentuate at sexual maturity. Individuals with lobes of 20 mm^2 or over were considered sexually mature because they contained loose or "running" milt.

Testis development was greatest during the period September to January (Fig. 6).

Of the males examined during the spawning period 50% were mature at lengths of 160–169 mm, and nearly all 170 mm and greater were mature:

Size class (*mm*)	120–129	130–139	140–149	150–159	160–169	170–179	180–189	190–199	200–209	210–219
% mature	0	0	23.8	38.2	51.0	92.6	76.2	94.6	100	100
No. examined	9	7	12	21	14	13	13	24	11	6

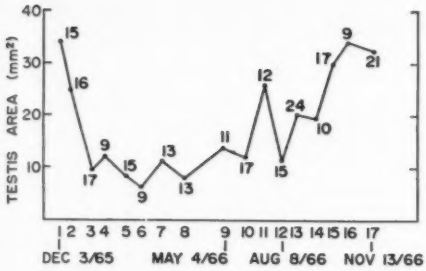

FIG. 6. Annual variation in average surface area (mm²) of one testis lobe of *L. pacifica*. Numerals above points indicate the numbers of males examined in the samples that were taken on dates shown in Table 1.

Females — The ovary of *L. pacifica* is a median unpaired structure which lies in the posterodorsal part of the body cavity. Ovarian membranes are continuous with the oviduct. In the immature condition ovarian structure was recognizable microscopically, but individual eggs could not be discerned. Females containing large (>2 mm diam) eggs were considered sexually mature. Large (>2 mm diam) eggs are yellow-orange, whereas medium (1–2 mm diam) and small (.2–1 mm diam) eggs tend to be light yellow.

The average complement of medium eggs increased in February in 1966, levelled off until about late August, then decreased (Fig. 7). In August large

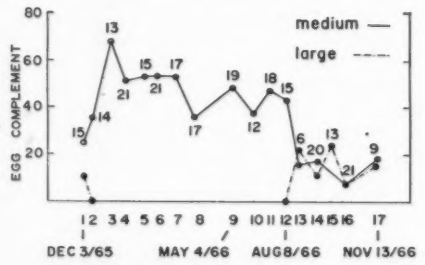

FIG. 7. Annual variation in average complements of medium (1–2 mm diam) and large (>2 mm diam) eggs of *L. pacifica*. Numerals above points indicate the numbers of females examined in the samples that were taken on dates shown in Table 1.

eggs began to appear and ripe females were found until late November. Large eggs were also present in samples taken in December 1965. The mature eggs of *L. pacifica* averaged 5 mm in diameter; the average complement of large eggs was 30.4 (range 7–52) in 51 individuals examined.

Of the females 50% were mature at lengths of 150–159 mm:

Size class (*mm*)	120–129	130–139	140–149	150–159	160–169	170–179	180–189
% mature	0	18.0	42.5	63.0	74.8	97.6	67.3
No. examined	10	11	19	19	20	15	3

Anatomical examinations of reproductive structures suggested that *L. pacifica* is oviparous. Evidence of modifications for internal fertilization or incubation, which would suggest viviparity or ovoviviparity, was not found. Some North Atlantic zoarcids, for example *Lycodes* spp. (Jensen, 1904) and *Macrozoarces americanus* (White, 1940), are oviparous, but *Zoarces viviparus* of European waters is viviparous according to Bretschneider and deWit (1947).

TABLE 4. Average percent abundance of food items[a] in digestive tracts of *L. pacifica* collected December 1965–November 1966 at outer Burrard Inlet. Twenty digestive tracts from each of the samples collected on the dates shown in Table 1 were examined.

Sample no.	Total no. items	A	B	CO	CU	DRA	DN	DRM	G	O	PE	PSP	PSS
1	160	11.9	25.6	0	2.5	0	0	0	0	1.2	4.4	45.6	8.2
2	156	18.6	42.3	0	3.2	0	0	0	0.6	1.3	5.8	23.1	5.1
3	244	5.3	68.8	0	2.5	0	1.2	0	1.6	2.8	8.2	6.9	2.5
4	245	2.8	50.2	0	0.4	0	0	0	1.6	0	2.8	36.7	4.9
5	209	13.8	44.9	0	0.5	0	0.9	0	0.9	1.4	3.4	31.1	2.8
6	301	17.6	41.8	0	0.3	0	0.3	0	0	0	4.6	15.3	3.9
7	188	20.7	32.5	0	7.9	0	0	0	4.3	2.1	9.6	22.9	0
8	305	22.3	49.8	0	2.6	0	0.3	0	0	0	3.9	18.0	1.9
9	838	0.5	84.4	0	0	0	0	0	1.2	0.3	4.3	9.4	0
10	464	6.3	65.9	0.4	0.6	0	0.2	0	0.4	0	0.4	18.3	7.3
11	430	2.2	72.4	0	0.6	0	0	0	0.2	0	2.4	17.8	4.2
12	290	1.4	66.9	0	0	1.4	0	21.7	3.1	0	2.1	4.5	0
13	282	13.9	47.5	0	0.7	0.4	0	0	0.4	0	2.5	28.4	6.4
14	347	2.9	83.8	0	1.2	0.6	0	3.5	0	0.8	4.0	3.2	0
15	90	6.7	47.8	0	0	0	0	0	0	2.2	2.3	35.6	4.4
16	352	42.6	37.2	0	0.3	0.9	0	0	0.9	0.6	0.9	16.2	0.6
17	87	6.9	62.1	0	2.3	0	0	0	0	1.1	1.1	22.9	3.4
Avg		11.5	54.3	0.02	1.5	0.2	0.2	1.5	0.9	0.8	3.5	20.9	3.3

[a]A = Amphipoda; B = bivalve molluscs; CO = Copepoda; CU = Cumacea; DRA = Decapoda, Reptantia (adults); DN = Decapoda, Natantia; DRM = Decapoda, Reptantia (megalops stage); G = gastropod molluscs; O = Ostracoda; PE = Polychaeta, Errantia; PSP = Polychaeta, Sedentaria (*Phyllochaetopterus* spp.); PSS = Polychaeta, Sedentaria (*Sternapsis* spp.).

The low fecundity and large eggs suggest that *L. pacifica* tends its young. This possibility is supported by indirect evidence provided by Dr E. W. Fager, Scripps Institution of Oceanography, La Jolla, California (personal communication) who observed *L. pacifica* from a submersible off southern California in 1964 and 1965. During dives in fall and winter he commonly observed *L. pacifica* in male and female pairs. On one occasion he observed a pair clearing a patch of sediment. It appeared as if a "burrow" was being constructed.

FOOD ITEMS

Bivalves, tubeworms, amphipods, free-living annelids, and sternapsids occurred most abundantly in the intestinal tracts of *L. pacifica* (Table 4). More complete analyses of these data may be found in Levings (MS, 1967). The species apparently feeds primarily on soft-bottom benthos, but detritus and sediments were found in all individuals. Several of the most abundant bivalves were: *Yoldia ensifera*, *Y. amygdalea*, *Macoma carlottensis*, and *Axinopsis sericatus*.

ACKNOWLEDGMENTS

The study was carried out under the auspices of Dr N. J. Wilimovsky, Institute of Fisheries, University of British Columbia, and was supported in part by grants to him. I would like to acknowledge the cooperation and help of Mr T. H. Butler, Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C., and Messrs K. Tanaka and J. Kondo, commercial shrimp fishermen, for collecting *L. pacifica*; Mr K. Freeman for construction of the figures; and Dr I. McTaggart-Cowan, Faculty of Graduate Studies, University of British Columbia, for identification of several bivalves. The manuscript was constructively reviewed by W. A. Kennedy and S. J. Westrheim, both of the Fisheries Research Board, Biological Station, Nanaimo, B.C. The manuscript was completed during the tenure of a Dalhousie Scholarship.

REFERENCES

BAYLIFF, W. S. MS, 1954. A review of the Zoarcidae of the northeastern Pacific Ocean. M.S. Thesis. Univ. Washington, Seattle, Wash. 189 p.

BRETSCHNEIDER, L. H., AND D. DEWIT. 1947. IV. The cyclic changes in the sexual endocrine organization of non-mammalian vertebrates. Monographs Progr. Res. Holland 11: 117–147.

BUTLER, T. H. MS, 1949. The status of the pink shrimp (prawn) *Pandalus borealis* Kroyer in the commercial shrimp fishery of English Bay, B.C. B.A. Thesis. Univ. British Columbia, Vancouver, B.C. 38 p.

CLEMENS, W. A., AND G. V. WILBY. 1961. Fishes of the Pacific coast of Canada. (2nd ed.) Bull. Fish. Res. Bd. Canada 68. 443 p.

GREGORY, W. K. 1933. Fish skulls: a study of the evolution of natural mechanisms. Trans. Am. Phil. Soc. (N.S.) 23: 75–481.

HOLLISTER, H. J. MS, 1960. Classification of seawater temperatures and salinity on the Pacific coast of Canada, 1915–59. Fish. Res. Bd. Canada, MS Rept. Ser. (Oceanog. Limnol.) 68. 139 p.

IRIE, T. 1960. The growth of the fish otolith. J. Fac. Fish. Animal Husbandry Hiroshima Univ. 3: 203–221.

JENSEN, S. 1904. Monograph of the Lycodids of northern Europe and Greenland. Danish Ingolf Exped. 2: 1–151.

LEVINGS, C. D. MS, 1967. Aspects of the life history of *Lycodopsis pacifica* (Collett) 1879. M.Sc. Thesis. Univ. British Columbia, Vancouver, B.C. 81 p.

MARTIN, W. R. 1949. The mechanics of environmental control of body form of fishes. Publ. Ont. Fish. Res. Lab. 70: 81 p.

RICKER, W. E. 1958. Handbook of computations for biological statistics of fish populations. Bull. Fish. Res. Bd. Canada 119. 300 p.

STEEL, R. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Toronto, Ont. 481 p.

WALDICHUCK, M., J. R. MARKETT, AND J. H. MEIKLE. MS, 1968. Fraser River estuaries, Burrard Inlet, Howe Sound, and Malaspina Strait. Physical and chemical oceanographic data. Vol. I and II, 1957–66. Fish. Res. Bd. Canada, MS Rept. Ser. 939. 554 p.

WHITE, H. C. 1940. The nesting and embryos of *Zoarces anguillaris*. J. Fish. Res. Bd. Canada 4: 337–338.

Final Observations on the Survival of Planted Lake Trout in South Bay, Lake Huron[1]

J. C. BUDD[2]

Research Branch, Ontario Department of Lands and Forests
Maple, Ont.

AND

F. E. J. FRY AND P. S. M. PEARLSTONE

Department of Zoology
University of Toronto, Toronto, Ont.

BUDD, J. C., F. E. J. FRY, AND P. S. M. PEARLSTONE. 1969. Final observations on the survival of planted lake trout in South Bay, Lake Huron. J. Fish. Res. Bd. Canada 26: 2413–2424.

Yearling lake trout were planted in South Bay, Lake Huron, an area with a sea lamprey population, each year from 1949 to 1955 with the exception of 1950. Fish were recaptured in pound and gillnets. Those recaptured in pound nets were tagged and released. Data from recaptures of tagged fish have been used to calculate yearly population estimates that were used to determine the annual mortality of certain year-classes. The fish were also examined for lamprey wounds and scars and the results tabulated according to the age of the fish.

Before age IV annual mortality of South Bay lake trout does not appear to differ significantly from annual mortalities observed in lake trout populations in the absence of sea lampreys. Thereafter, annual mortality rates rise sharply and the population disappears at the age of VI or VII. No spawning population was maintained.

Prior to age III no lamprey marks were observed on recaptured lake trout. From ages III to V the percentages of fish bearing lamprey marks increased from 5.2 to 38.8. This trend may continue through age VI but too few data are present to be certain. Before age VI there appears to be a direct correlation between annual lamprey marking rates and annual natural mortality rates.

Received September 27, 1968

INTRODUCTION

THIS IS the final paper of three concerning the survival of a series of plants of yearling lake trout into South Bay where a sea lamprey population had eliminated the native stock. South Bay is a narrow-mouthed bay approximately 15 miles long, in Manitoulin Island, northern Lake Huron. Planting began in 1949 and was continued yearly until 1955 with the exception of 1950. All

[1]Contribution no. 67-20 of the Ontario Department of Lands and Forests, Research Branch, Maple, Ont.

[2]Deceased September 15, 1962.

Printed in Canada (J1234)

fish planted were marked by the removal of the adipose fin. Planted fish were recaptured in pound and gillnets. Those taken in the pound nets were tagged and released. The gillnet fishery followed the pound-net fishery and recaptures of the tagged fish were recorded together with the total catch. The last of the planted lake trout was captured in 1960.

Fry and Budd (1958) presented the results of the plantings through 1956, and Budd and Fry (1960) those through 1957. This paper will complete documentation of observations on recovery of planted fish and on evidence of lamprey attacks on them, as well as providing further population estimates and mortality data made possible by increased gillnet fishing in the later years of the experiment.

METHODS

South Bay comprises two basins separated by a moderately broad constriction. The outer basin is quite shallow (with an average depth of 7 fath) and the inner basin is considerably deeper. Five pound nets were employed throughout the experiment and were set each year in the same locations in the outer basin (locations A, A¹, B, C, E, Fig. 1). The pound-net fishery extended from May until early July. Lake trout captured in the pound nets were tagged in front of the dorsal fin with a celluloid streamer tag (Fry, 1953) and released. After the pound nets had been removed, gillnets were fished in various locations throughout the bay (mainly in the inner basin) until mid-autumn. In the early years of the experiment there was little gillnet fishing

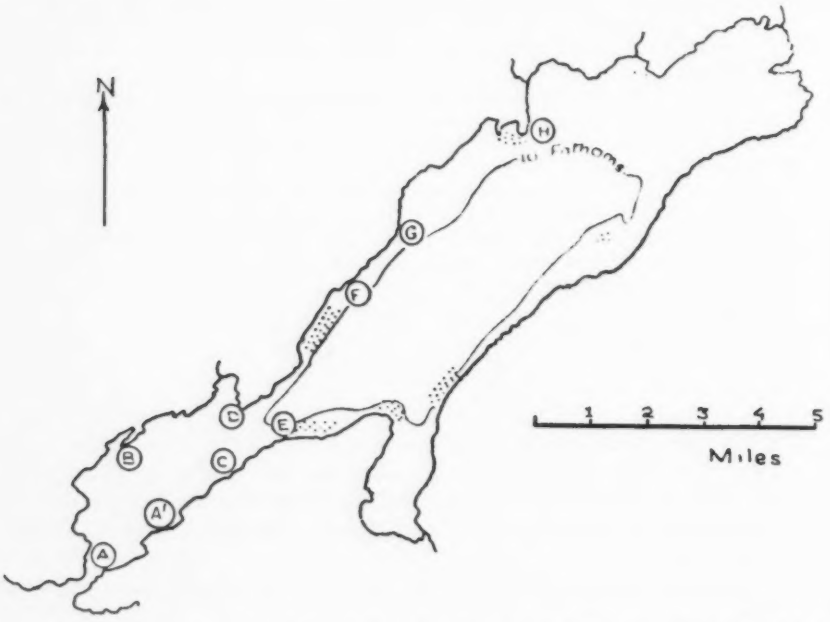

FIG. 1. South Bay; the various letters indicate locations at which pound nets have been fished. Stippled areas are former trout lake spawning beds. Modified from Fry (1953).

since it was hoped that the sport fishery for lake trout would be revived, and that a creel census would provide information on lake trout recaptures. In 1956, by which time it had become evident that anglers were not again fishing actively for lake trout, the gillnet fishery was increased. This increase resulted in a considerable number of gillnet recaptures of tagged fish throughout the later years of the experiment.

RESULTS AND DISCUSSION

Recovery of Planted Fish

Table 1 shows the numbers of lake trout planted during the course of the experiment together with the numbers of subsequent recaptures. Any records of tagged fish captured a second time have been omitted from the

TABLE 1. Summary of lake trout catches in South Bay over the years 1952–60. Tagged fish recaptured have not been included. Numbers in italics transcribed from Fry and Budd (1958). Numbers in parentheses are unmarked fish; all fish taken in 1958 were recorded as "planted," i.e., assumed to be finclipped.

Year-class	No. planted (*100's*)	Age II	III	IV	V	VI	VII	Total	% unmarked
1947	*0*				*0(4)*	*1(0)*	*1(1)*	7	70
1948	*71*			*73(16)*	*90(14)*	*79(2)*		274	12
1949	*0*		*2(0)*	*0(4)*	*2(2)*			10	60
1950	*101*	*2(0)*	*12(3)*	*85(8)*	*20(2)*	*9(1)*		142	10
1951	*231*	*0(1)*	*24(6)*	*25(4)*	*149(22)*	5(0)		236	14
1952	*45*	*17(5)*	*63(14)*	*386(40)*	86(0)	2(0)		613	10
1953	*184*	*30(5)*	*193(18)*	314(5)	45(0)	9(0)		619	4
1954	*157*	*140(13)*	866(2)	942(0)	351(3)	34(3)		2354	1
Total		213	1203	1902	790	145	2	4255	

tabulation. Although records of unclipped fish taken are given separately, the small numbers obtained would seem to indicate that these were not native fish, but rather fish with regenerated fins on which finclips were not recognized.

The new data in Table 1 substantiate the earlier observations that very few fish survived to age VI and virtually none to age VII. Hence there is continuing evidence that the lake trout population in South Bay cannot survive to spawn in the presence of the present population of sea lamprey. Taking the total captures at a given age over all years, there was a 58% reduction in the lake trout between ages IV and V and 82% between ages V and VI.

Population Estimates

Fry and Budd (1958) and Budd and Fry (1960) have presented Schnabel population estimates (DeLury, 1951) based on the pound-net fishery. Increased gillnet catches from 1956 to 1960 have enabled calculation of further estimates (of the Petersen type) on the basis of recaptures in gillnets of fish previously

tagged within the same year in pound nets. In addition, a third series of estimates (also Petersen) has been included. These latter estimates were based on total recaptures in year N+1 of age-groups that were tagged in the fishery in year N. Since the 1954 year-class was the last planted, recruitment was essentially complete by 1957. Thus in practice a correction for net selection had only to be made for 1956. The second Petersen estimate for that year was made by eliminating from the calculations the few fish tagged at age II in 1956 and all fish captured in 1957 at age III. Table 2 lists the three series of estimates. The values of the two Petersen estimates agree well, whereas the corresponding Schnabel estimates are consistently lower.

The movements of the South Bay lake trout (Fry, 1953) possibly provide an explanation for the discrepancy between the Schnabel and Petersen estimates. In the spring, the lake trout are to be found throughout the shallow water of the outer basin and along the northwestern shore of the inner basin. In early summer, the fish retreat below the thermocline, and until fall are almost exclusively located within the inner basin below 10 fath. The continuation of an almost total absence of lake trout tag returns from Lake Huron outside the bay (only two tags have been returned since 1955) indicates that the planted lake trout were confined to South Bay as were the native population before them. Therefore, virtually the entire population is to be found in the inner basin during the summer. Since all five pound nets upon which the present study is based were in the outer basin, they did not necessarily sample the total lake trout population.

The fish tagged in the outer basin do, however, join the remainder of the population in the inner basin and are presumably mixed with them at the time of the gillnet fishery. Fry (1953) has shown that tagged lake trout caught in the summer fishery came in equal proportions from groups tagged in the inner and outer basins. Thus, population estimates based on returns from the gillnet fishery may be expected to reflect the total population. The Petersen estimates based on tags extant for a year taken in the total fishery are, of course, much weaker and serve only as supplementary evidence.

The Petersen estimates given in Table 2 give a general picture of the size of the lake trout population in South Bay that is vulnerable to the gillnet fishery, which was carried on with a graded series of nets from $1\frac{1}{2}$ to $4\frac{1}{2}$" stretched mesh and thus probably equally efficient in the capture of a wide size range of fish.

To estimate mortalities and survival from planting, Petersen estimates were made for each age-group where possible. These estimates together with other published estimates of the strength of age-groups are given in Table 3. It will be noted, if the comparison is made, that the aggregate estimate in Table 2 is often greater than the sum of the individual estimates in Table 3. The difference is due in some instances to the omission there of estimates at age II and otherwise to smaller fish being more vulnerable to the gillnets than to the pound nets (cf. Fry, 1953) from which the tagged fish were released. From the data in Table 3, some of the progress of the individual year-classes

TABLE 2. Summary of population estimates based on recaptures of tagged fish released from pound nets.

Year	Tags released	Schnabel estimate (based on recaptures in pound nets)	Petersen estimate year N			Petersen estimate year N+1		
			No. recaptures with tags	No. caught without tags	Estimate	No. tag recaptures year N+1	No. caught without tags year N+1	Estimate
1956	181	–[a]	25	575	4160	9	323	5960
1957	567	2550	33	741	12730	48	1105	13050
1958	728	2780	53	307	4220	54	395	5330
1959	205	1590	21	173	1700	3	36	2460
1960	34	–[a]	5	8	50	–	–	–

[a]Insufficient data for estimate.

TABLE 3. Estimates of the strengths of various year-classes of lake trout in South Bay.

Age	Year-class							
	1944	1945	1948	1950	1951	1952	1953	1954
III								
Tags released								278
Tags recaptured								19
Unmarked								567
Estimate								8266
IV								
Tags released						94	179	674
Tags recaptured						14	11	42
Unmarked						279	142	313
Estimate	16700[a]	3200[a]		310[b]	80[b]	1873	2310	5022
% mortality								39
V								
Tags released					52	57	30	204
Tags recaptured					10	3	3	16
Unmarked					93	33	15	166
Estimate	10800[a]	1000[a]	1400[b]	160[b]	484	627	150	2116
% mortality	36	68		48	?	67	93	58
VI								
Tags released							1	
Tags recaptured								
Unmarked							9	
Estimate	3000[a]	125[a]	700[b]				–[c]	
% mortality	72	88	50	100				
VII								
Estimate	475[a]							
% mortality	85		100					

Avg. % mortality: age V — 62
age VI — 78
age VII — 92

[a]Prorated Schnabel estimates (Fry and Budd, 1958).
[b]Estimate from catch per unit of effort in pound nets (Fry and Budd, 1958).
[c]Insufficient data for estimate.

can be followed. The first two year-classes for which data are available (1944 and 1945) comprised native fish; the remainder were planted fish.

As has been pointed out above, Schnabel estimates of the South Bay lake trout do not reflect the total population. The earlier data in Table 3 are Schnabel estimates or are derived from them from data on the catch per unit effort in pound nets (Budd and Fry, 1960, fig. 1).

For the years subsequent to 1950, the tagging data upon which the estimates are based have been summarized in Table 3. Wherever consecutive yearly estimates have been made for a given year-class, the percentage mortality from one year to the next has been noted.

Figure 2 illustrates the population data that have been listed in Table 3. Since the pound nets used in the experiment did not catch small trout, few data were collected prior to age IV. Therefore, the first estimate calculated for each year-class has been joined by a dotted line to the number known to have been planted at age I (Table 1).

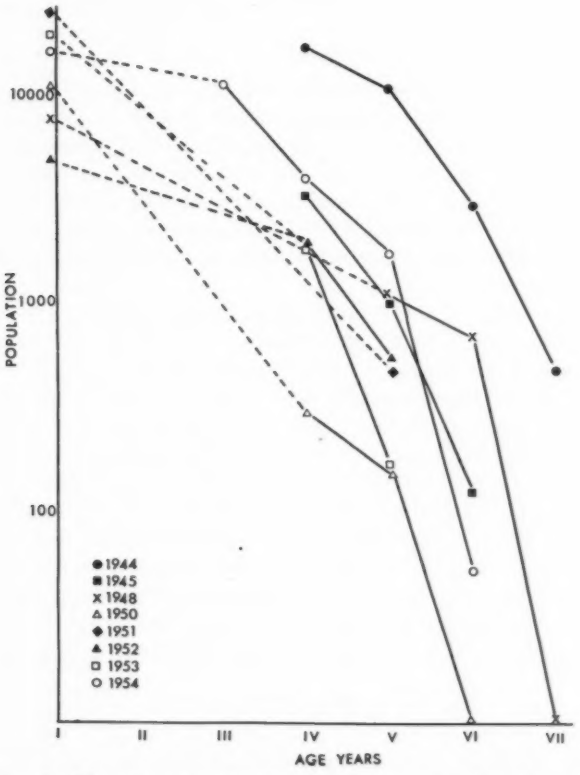

FIG. 2. Mortality curves based on successive population estimates for various year-classes of lake trout in South Bay, Manitoulin Island.

For the 1954 year-class (the only year-class for which it was possible to calculate population estimates at age III), the extrapolation back to the number planted shows an early mortality similar to the annual natural mortality rate of lake trout in the absence of a lamprey population as observed in Lake Manitou (Budd et al., 1968), Lake Opeongo (Paloheimo, 1958), and Cold Stream Pond (DeRoche and Bond, 1955), i.e., about 20%. Two other year-classes (1948 and 1952) show a similar trend in early mortality. It seems safe, therefore, to propose that the South Bay lake trout population was not seriously affected by the sea lamprey before age IV.

After age IV, the mortality became more serious, as Fig. 2 demonstrates. As is suggested by Fry and Budd (1958) the delayed increase in mortality of the 1948 year-class until age VI is likely due to the unusually slow growth of this group since mortality was observed to increase with the size of the fish. Mortality of the lake trout is closely related to evidence of lamprey attacks on fish examined, as is discussed below.

Lamprey Predation and Mortality

All lake trout, both those released with tags and those examined in the laboratory, were examined for lamprey wounds and scars. The earlier observations are published in Fry (1953), Fry and Budd (1958), and Budd and Fry (1960). Few lamprey marks were seen on trout in 1947 and 1948 and over all years there has been little evidence of lamprey attacks on lake trout less than 17 or 18 inches long. Since 1949 a substantial fraction of the larger fish examined have been marked. The lamprey marking data for 1950 to 1960 are summarized by age in Table 4.

No trend is evident in the proportion of lamprey-marked trout over the years 1950–60 but the relation of evidence of attack to age remains strong. No lamprey marks were found on lake trout of age II. At age III, 5% of the fish examined bore lamprey marks and at age IV 23% were marked. The proportion of marked fish reached a peak at age V of 39%. In the total sample at age VI only 26% were marked but if the 1950 catch, which made up more than $\frac{3}{4}$ of the total, is removed 37% were marked at age VI.

A comparison of evidence of lamprey predation with the annual mortality is given in Fig. 3. It was considered that such a relation should be sought between the proportion of lamprey marks added from year N-1 to year N and the natural mortality during the same period. It was expected that lamprey marks would accumulate in the population from year to year. To correct for such an accumulation the competitive equation (Ricker, 1958) was applied in the form:

$$M_1 + M_2 - M_1M_2 = T$$

where T = the fraction observed bearing lamprey marks in year N; M_1 = the fraction of the population bearing marks as the result of attacks in year N-1; M_2 = the fraction of the population bearing marks as the result of attacks in year N.

Thus, taking the 1954 year-class as an example, the marks to be attributed to attacks during age IV would be, from Table 4, years 1957 and 1958:

$$.04 + M_2 - .04\ M_2 = 0.19$$
$$M_2 = 0.16$$

A correction was also made for fishing mortality to derive the annual natural mortality rate as follows.

TABLE 4. Summary of observations of lamprey wounds and scars on lake trout in South Bay. Data for 1957 are reprinted from Budd and Fry (1960).

Year		Age					
		I	II	III	IV	V	VI
1950	No. fish			1		183	670
	No. marked					25	161
	No. marks					31	198
	% marked					13	24
1951	No. fish		1	4	1	14	36
	No. marked				1	1	7
	No. marks				1	1	9
	% marked				100	7	19
1952	No. fish		14	8	74	5	4
	No. marked						1
	No. marks						1
	% marked						25
1953	No. fish		25	24	26	120	1
	No. marked			2	1	17	
	No. marks			3	1	17	
	% marked			8	3	14	
1954	No. fish	2	22	34	95	5	85
	No. marked			1	4		15
	No. marks			1	4		20
	% marked			3	4		17
1955	No. fish		39	89	26	23	
	No. marked			12	12	12	
	No. marks			17	13	19	
	% marked			13	46	52	
1956	No. fish		93	133	382	145	8
	No. marked			9	111	70	3
	No. marks			10	150	104	6
	% marked			6	29	48	37
1957	No. fish			908	341	103	8
	No. marked			38	130	68	5
	No. marks			45	192	95	10
	% marked			4	38	66	63
1958	No. fish				980	49	7
	No. marked				189	35	5
	No. marks				221	60	7
	% marked				19	71	71
1959	No. fish					406	8
	No. marked					181	6
	No. marks					248	12
	% marked					45	75
1960	No. fish						38
	No. marked						24
	No. marks						36
	% marked						63
Total	No. fish	2	194	1201	1925	1053	865
	No. marked			62	448	409	227
	No. marks			76	582	575	299
	%marked			5.2	23.3	38.8	26.2

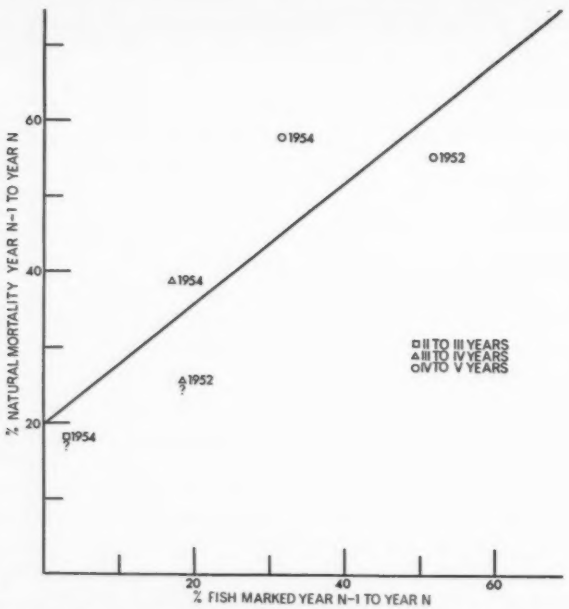

FIG. 3. Relation between lamprey marks on surviving fish and annual natural rate in South Bay lake trout. The queries associated with the two points for the 1954 and 1952 year-classes indicate that the mortality associated with them is the mean over the period from planting to the age observed. The lamprey mark data associated with the points for the 1944 and 1945 year-classes, ages IV–V, are estimates from Fry (1953), Tables 4 and 6.

Again from the competitive mortality formula:

$$M + N - MN = T$$

where M = the annual fishing mortality rate; N = the annual natural mortality rate; and T = the total mortality rate.

It can be shown that where C is the total annual catch and D the total annual death from natural mortality that

$$\frac{C}{D} = \frac{M(1 - N)}{N(1 - M)} = A.$$

From the two equations above

$$M = \frac{-(T - 1)(1 - A) \pm \sqrt{((T - 1)(1 - A))^2 - 4A^2T}}{2A}$$

one root being real.

To solve the equation, C is taken as the total of tagged plus untagged fish recaptured for a given age in a given year, rounded off to the nearest 10 above the number given. The small correction is made to account for fish killed in the pound nets.

$$D = (P_1 - P_2) - C$$

where P_1 is the population estimate associated with C; and P_2 the appropriate population estimate in the following year; and

$$T = \frac{C + D}{P_1 - P_2}.$$

Estimates of lamprey marking and natural mortality were made in instances where the smaller catch concerned was at least 50 fish. Only instances in which Petersen estimates were made by catches in the graded series of gillnets were considered.

Bearing in mind the uncertainty associated with the individual population estimates, the points in Fig. 2 show a trend similar to that described by the line drawn. The line given in the figure was not fitted to the points but is a straight line drawn from 0 lamprey predation, 20% annual mortality (see above) to 100% lamprey predation, 100% annual mortality.

On the basis of the grand means in Table 4 — even allowing for the 1950 data — there would be no lamprey marks added from age V to age VI. It seems probable that the anomaly arises from small samples but it is possible that as fish get larger they are less likely to survive a lamprey strike. Whatever the explanation, the data in Fig. 2 illustrate only conditions for lake trout in South Bay age V and younger.

CONCLUSIONS

1. Planted lake trout in South Bay are able to survive up to age IV along with the present lamprey population, but are not able to sustain themselves long enough to maintain a spawning population.

2. During the years prior to age IV, lake trout mortality does not appear to be seriously affected by the sea lamprey.

3. Prior to age VI, there appears to be a direct correlation between annual lamprey marking rates and annual natural mortality rates.

ACKNOWLEDGMENTS

We are most grateful to Mr J. B. Smith who read the lake trout scales on which this study is based. The field work was carried out by the staff of the South Bay Fisheries Station, at that time under the capable foremanship of the late W. I. Chapman. The records of the station have for some years been in the charge of Mrs G. W. Bennett. Without her meticulous attention to detail we would not have been able to complete this study.

REFERENCES

BUDD, J. C., AND F. E. J. FRY. 1960. Further observations on the survival of yearling lake trout planted in South Bay, Lake Huron. Canadian Fish Culturist 26: 7–13.

BUDD, J. C., F. E. J. FRY, AND J. B. SMITH. 1968. Survival of marked lake trout in Lake Manitou, Manitoulin Island, Ontario. J. Fish. Res. Bd. Canada 25: 2257–2268.

DELURY, D. B. 1951. On the planning of experiments for the estimation of fish populations. J. Fish. Res. Bd. Canada 8: 281–307.

DEROCHE, STUART E., AND LYNDON H. BOND. 1955. The lake trout of Cold Stream Pond, Enfield, Maine. Trans. Am. Fish. Soc. 85: 257–270.

FRY, F. E. J. 1953. The 1944 year class of lake trout in South Bay, Lake Huron. Trans. Am. Fish. Soc. 82: 178–192.

FRY, F. E. J., AND J. C. BUDD. 1958. The survival of yearling lake trout planted in South Bay, Lake Huron. Canadian Fish Culturist 23: 13–21.

PALOHEIMO, J. E. 1958. A method of estimating natural and fishing mortalities. J. Fish. Res. Bd. Canada 15: 749–758.

RICKER, W. E. 1958. Handbook of computations for biological statistics of fish populations. Bull. Fish. Res. Bd. Canada 119. 300 p.

Progressive Changes in the Ventral Aorta of the Carp, *Cyprinus carpio*

JOSEPH T. EASTMAN

Department of Zoology
University of Minnesota, Minneapolis, Minn. 55455, USA

EASTMAN, JOSEPH T. 1969. Progressive changes in the ventral aorta of the carp, *Cyprinus carpio*. J. Fish. Res. Bd. Canada 26: 2425–2430.

Early arteriosclerosis was found in the curved region of the ventral aorta of the carp, *Cyprinus carpio* Linnaeus, older specimens showing a greater degree of involvement than younger specimens. The progressive pathological changes are attributable to the length of time that a particular set of hemodynamic conditions, resulting in a suction on the inner wall of the arterial curve, have been operating.

EASTMAN, JOSEPH T. 1969. Progressive changes in the ventral aorta of the carp, *Cyprinus carpio*. J. Fish. Res. Bd. Canada 26: 2425–2430.

De l'artériosclérose dans les premiers stages a été trouvée dans la région courbée de l'aorte ventrale de la carpe, *Cyprinus carpio* Linnaeus, les spécimens plus agés montrant un degré de complication plus avancé que les spécimens plus jeunes. Les changements pathologiques progressifs sont attribuables à la durée d'un assortiment particulier de conditions hémodynamiques, résultant en une succion de la cloison interne de la courbe artérielle.

Received May 13, 1969

INTRODUCTION

IT HAS BEEN SUGGESTED (Lankester, 1870; Comfort, 1964) that teleosts do not exhibit senescence as do the higher vertebrates. To date there is little histological evidence of progressive vascular changes in fishes. Vastesaeger et al. (1965) examined teleosts from 10 families and found focal fibro-elastic intimal proliferations in the coronary arteries of 18 specimens in a sample of 101. In their study, atherosclerotic (i.e., fatty) lesions were present only in the bluefin tuna fish. The question to be examined in this paper is: can progressive changes be histologically distinguished in the ventral aorta of the carp and, if so, can an explanation for the changes be found?

The ventral aorta (Fig. 1, 2A) was suspected to show progressive changes because of its form (i.e., an obtuse angle of 150°) and the particular set of hemodynamic conditions that are the result of this curve.

MATERIALS AND METHODS

Carp, *Cyprinus carpio* Linnaeus, were obtained from the L. F. Miller Fisheries (Lakeland, Minn.) and from the Minnesota Department of Conservation. All specimens were netted in Lake St. Croix (Washington Co., Minn.), held in pens in the lake and secured for study while still alive. The sample consisted of 50 carp ranging in age from 1 to 10 years (i.e., year-classes 0–IX), in weight from 4.0 g to 10.4 kg, and in total length from 5.0 cm to 88.3 cm. Ages were determined by reading scale annuli.

Printed in Canada (J1473)

Immediate postmortem fixation of the heart–ventral aorta–afferent branchial artery complex was in calcium formalin with subsequent dehydration in ethanol, clearing in xylene, and embedding in paraffin. Transverse and longitudinal sections of vessels were cut at a thickness of 8 μ and most of them were stained with Weigert's elastica for the simultaneous demonstration of elastin, collagen, and muscle. Other sections were stained with orcinol–new fuchsin (for demonstration of elastic tissue), Mallory's triple, hematoxylin and eosin, toluidine blue O (for mast cell granules), or Alcian blue (for mucopolysaccharides). Mounting was in Permount.

To test for lipids, the arteries of some members of each year-class were fixed for 48 hr in a 1% Osmium tetraoxide solution and then carried through the usual paraffin methods except that clearing was in cedarwood oil, not xylene. In addition, staining with Sudan black B and oil red O was performed on some 9-μ paraffin sections that were subsequently mounted in Apathy's gum syrup. To check for superficial intimal lipid deposits, whole ventral aorta–afferent branchial artery complexes were dissected and stained grossly with a Sudan IV solution. After differentiation in 70% ethanol, the vessels were cut open for inspection under a dissecting microscope.

When referring to the regions of the pharyngeal arches, the terminology used in this paper is based on that of Chu (1935).

DESCRIPTIONS OF PROGRESSIVE CHANGES

The dorsal aorta in the region of the aortic angle exhibited marked progressive changes (Fig. 2B–F). Grossly visible thickening, most pronounced in older year-classes, on the inner surface of the dorsal wall (i.e., the concave side of the aortic angle curve) with concomitant histological alteration of all three arterial layers was found. The ventral wall of the ventral aorta in the aortic angle region also showed a higher frequency of changes in older specimens although the changes were not so striking as those seen in the dorsal wall.

Year-class 0 — The dorsal aortae of carp belonging to this year-class appeared free of significant changes; no differential wall thicknesses were apparent and all three layers appeared normal.

Year-class I — The carp in this year-class had begun to show dorsal wall changes (Fig. 2B). The endothelium of the tunica intima was disrupted as was the internal elastic membrane, which was also slightly thickened and hyperplastic along the middle one-third of the wall. The tunica media was two to three times thicker on the dorsal side than on the ventral side. The elastic laminae of the media were arranged in the expected concentric pattern. Single hyperplastic elasto-collagenous nodular lesions projected from the dorsal wall into the lumen. In addition, some of the peripheral elastic laminae

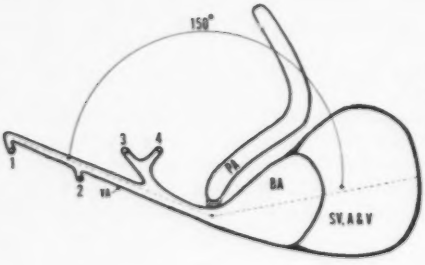

FIG. 1. Schematic diagram of the heart and related arteries of the carp — lateral view. The aortic angle of 150° is indicated. BA, bulbus arteriosus; PA, left pharyngeal arch; SV, A & V, sinus venosus, atrium & ventricle; VA, ventral aorta; 1–4, proximal parts of left afferent branchial arteries.

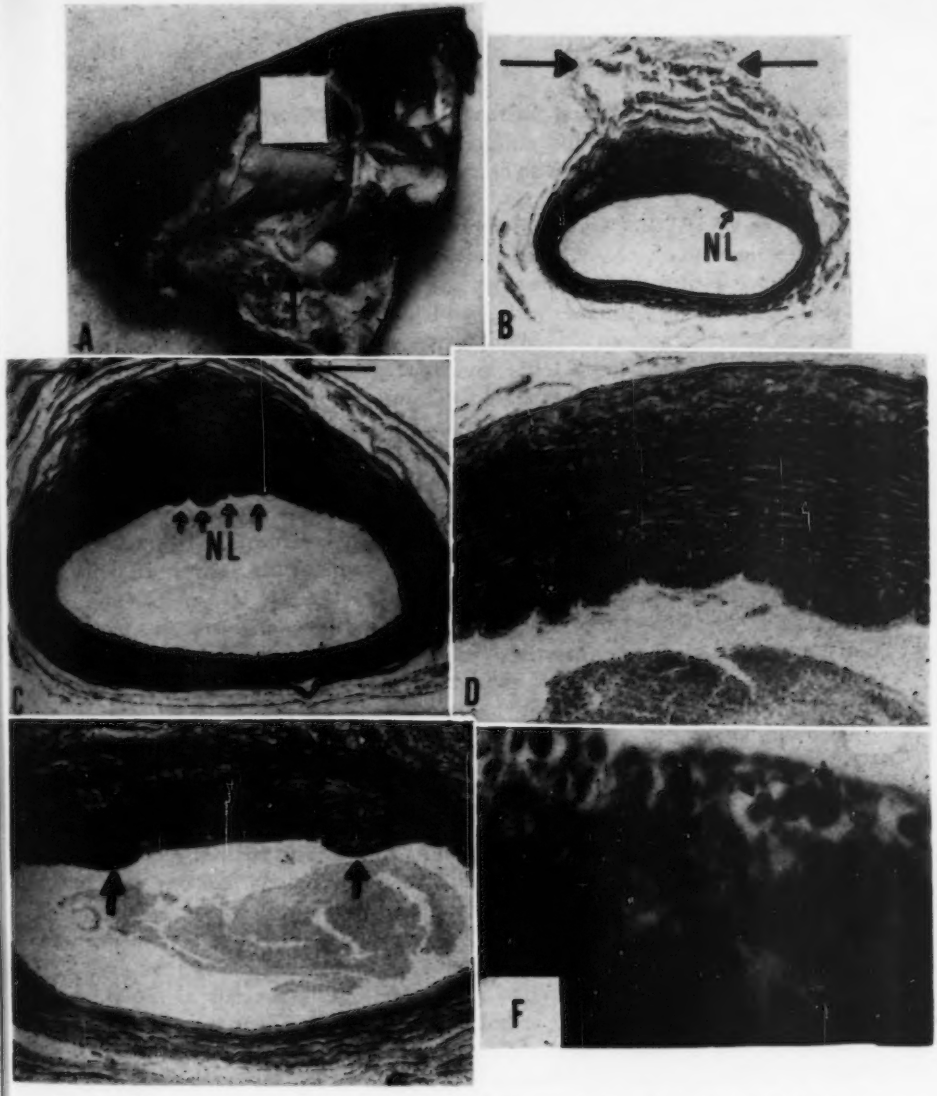

FIG. 2. (**A**) Lateral view of the head region of a 4.8-kg carp. Arrow indicates region of the vertex of the aortic angle. Tips of anterior limbs of pharyngeal arches project into the concavity of the aortic angle. White patch represents 2.54 cm². (**B**) Ventral aorta at the aortic angle region of a carp from year-class I. ×34. Weigert's elastica stain (elastin dark and collagen light). The thickened dorsal wall has one nodular lesion (NL). The arrows indicate the attachment of the dorsal wall to the tips of the anterior limbs of the pharyngeal arches. (**C**) Ventral aorta at the aortic angle region of a carp from year-class III. ×34. Weigert's elastica. The dorsal wall shows several small nodular lesions (NL) and a thickened media and adventitia. Horizontal arrows indicate the attachment of the dorsal wall to the pharyngeal arches. (**D**) Close-up of the dorsal wall of the ventral aorta at the aortic angle region of another specimen from year-class III. ×85. Weigert's elastica. Note the irregular arrangement of the elastic fibers in the lesions. (**E**) Ventral aorta at the aortic angle region of a carp from year-class VI. ×34. Weigert's elastica. The whole dorsal wall is excrescent and some distinct nodular lesions (arrows) are also distinguishable. Note the interlacing and irregularity of the elastic laminae in the extremely thickened dorsal wall media. Pockets of collagen (lightly stained areas) are also present in the dorsal wall media. A lightly staining 25-μ layer of subintimal collagen can be seen in the ventral wall. (**F**) Close-up of the intimal region of one of the nodules in E. ×850. Weigert's elastica. The endothelium is disrupted in places and the internal elastic membrane is not distinguishable. Mast cells (arrows) and fibroblasts are found in the intima along with some collagen. Note the small, irregularly arranged elastic fibers of the lesion in the media beneath the intima.

of the tunica media extended into the adventitia at acute angles to a tangent to the media–adventitia junction. The tunica adventitia was also thicker dorsally than ventrally, especially in the region of attachment to the pharyngeal arches (Fig. 2B).

Year-classes II and III — More advanced dorsal wall lesions were seen in specimens belonging to these year-classes (Fig. 2C, D). The tunica media beneath these nodular lesions was two to three times thicker than the ventral wall media and the nodules were not focal; they extended for about 5.0 mm on either side of the vertex of the aortic angle. Microscopically the nodules were seen to be covered by a thick intima (10–15 μ) that was abnormal in that it consisted mainly of collagen. The covering endothelium was disrupted and the endothelial cells did not exhibit the usual pavement disposition. Moreover, the internal elastic membrane was completely fragmented and scattered throughout the thickened intima. Toluidine blue O staining indicated the presence of mast cells in the intercellular spaces among the collagen fibers of the intima. The intimal collagen was probably produced by fibroblasts that migrated into the intima from the tunica media.

Year-classes IV–IX — In specimens from these year-classes the dorsal wall was excrescent in addition to showing discrete nodulation (Fig. 2E). The degree of involvement of the dorsal wall was greater in older than in younger year-classes but the basic patterns were similar. The dorsal wall media was three to four times thicker than that of the ventral wall and the same was true for the adventitia. Furthermore, the elastic laminae of the tunica media of the dorsal wall had completely lost their original concentric orientation and had become interlaced at oblique angles to each other. Pockets of collagen were found in the dorsal wall media, and the dorsal wall tunica intima had none of the characteristic features of a normal intima: the internal elastic membrane had fragmented and was dispersed; the endothelium was disrupted; and collagen fibers, fibroblasts, and mast cells had become the main intimal components (Fig. 2F).

The ventral wall of the aorta showed changes of a milder degree than those found in the dorsal wall. Members of year-classes I, II, and III had relatively normal ventral walls but specimens in year-class IV had a layer (15 μ thick) of subintimal collagen. In the older year-classes (V–IX) the subintimal collagen layer was 25 μ thick (Fig. 2E) and was accompanied by a split, frayed, and duplicated internal elastic membrane. The endothelium was, however, intact and normal.

The noncurved expanses of the ventral aorta anterior to the aortic angle were examined in the regions between the origins of the afferent branchial arteries. No differential wall thicknesses were noted in these straight spans in any of the year-classes. In members of the older year-classes (V–IX), however, some changes were found in the tunica intima; the internal elastic membrane was frayed, beaded, split, and discontinuous and, in some places, separated from the tunica media by intercellular spaces containing mast cells. Lipid staining was negative in all regions of the ventral aorta.

DISCUSSION

The progressive changes found in the ventral aorta of the carp are attributable to the length of time that a particular set of hemodynamic conditions have been operable in the vessel. The hemodynamics are, in turn, a reflection of the morphology (i.e., angles, bends, and attachments) of the vessel.

Texon (1963, 1967) has analyzed vascular hemodynamics and its role in the development of atherosclerosis. He has found that fluid flow conditions, as they exist in a cardiovascular system, conform with the classical laws of fluid dynamics. The changes found in carp arteries can be explained by applying these laws to blood flow.

Flow in vessels is usually streamline (i.e., laminar) below a critical velocity, above which it becomes turbulent. Turbulence may be found in large blood vessels in which the flow velocity is relatively high. Turbulence in the ventral aorta was not investigated in this study; it will be assumed that flow in this vessel is laminar. The pulsatile flow in vessels proximal to the gills does not alter the development of the low pressure zone at the aortic angle (see below).

The progressive changes found in the curved region of the ventral aorta can be explained by evaluating the hemodynamics of this region. In curvilinear motion (i.e., streamline flow in curves) the flow velocity is decreased and the lateral pressure is increased along the outer (i.e., convex) wall because of the vortex effect (Texon, 1967, p. 37). This effect also causes flow velocity to increase and lateral pressure to decrease along the inner (i.e., concave) wall (Fig. 3).

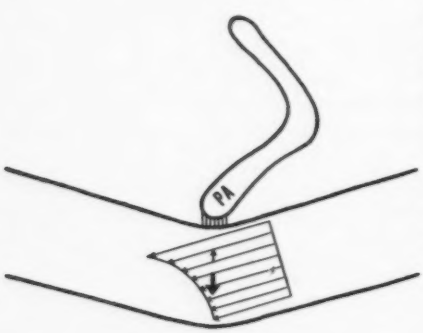

FIG. 3. A depiction of blood flow velocity and lateral pressure in the curved aortic angle region of the ventral aorta. The effects of pulsatile flow and separation with retrograde flow are intentionally omitted. The vortex effect results in decreased flow velocity and increased lateral pressure along the outer (i.e., convex) wall and increased flow velocity and decreased lateral pressure along the inner (i.e., concave) wall of the curve. Since the lateral pressure on the inner wall is not so great as that on the outer wall, a suction acts on the inner wall of the curve. The attachment of the dorsal wall to the anterior limbs of the pharyngeal arches is indicated. Thin vectors represent flow velocity. Thick, vertically orientated vectors represent lateral pressure. PA, left pharyngeal arch.

This decrease in lateral pressure produces a suction on the inner wall of the curve. The effects of suction are further pronounced when the inner wall of the curve is attached externally as the ventral aorta is at the aortic angle, where it is bound to the tips of the anterior limbs of the pharyngeal arches by a fibrous connection. The inner wall of the curve, being externally attached, is subject to even more strain since it cannot freely move toward the area of lower pressure. The effect of this suction is manifest in the histological alteration

of the dorsal wall components as evidenced by: thickening of all three arterial layers, disruption and fragmentation of the endothelium and internal elastic membrane, appearance of mast cells in the tunica intima, loss of the normal concentricity of the tunica media elastic laminae, interlacing of the tunica media elastic laminae, and penetration of the peripheral elastic laminae of the tunica media into the tunica adventitia.

Time, blood pressure, and proximity to the heart are important in determining the degree of progressive change found on the inner wall of the curve. Radius of curvature, lumen diameter, and velocity of flow determine the magnitude of the suction effect in a given blood vessel. A progressive sequence of changes, such as those found on the inner wall of the aortic angle curve of the carp, are indicative of the effect suction has had in this region.

The curve in the ventral aorta of the carp is due to the presence of the pharyngeal arches (i.e., hypertrophied fifth ceratobranchials). Since the anterior limbs of the pharyngeal arches preclude a straight course for the ventral aorta from the heart to the gills (Fig. 1), it is necessary for the ventral aorta to curve around the tips of the anterior limbs. Presumably this curve and its influence on the hemodynamics of the region is the primary causative factor in the development of the progressive changes found in the ventral aorta of the carp.

To test this hypothesis, histological examination was performed on the ventral aortae of eight lake trout, *Salvelinus namaycush* (Walbaum), a 28-kg paddlefish, *Polyodon spathula* (Walbaum), a 19-kg flathead catfish, *Pylodictis olivaris* (Rafinesque), and a 2-kg longnose gar, *Lepisosteus osseus* (Linnaeus). The paddlefish and catfish were both 20 years old. All these species lack well-developed pharyngeal arches and consequently the ventral aorta is not curved. No progressive changes such as those described for the aortic angle region of the carp were noted in any of these fishes.

The changes found in the outer (i.e., ventral) wall of the aortic angle curve in the carp of older year-classes (IV–IX) may be attributed to the relatively high lateral pressure that this wall has been subjected to. The formation of a thick, concentrically arranged layer of subintimal collagen to aid in buttressing the wall is indicative of the strain on the wall caused by the lateral pressure.

Progressive arterial changes, then, are demonstrable in a lower vertebrate previously thought not to senesce. The changes found in the carp arteries examined can be considered to be early or primary arteriosclerosis (i.e., a progressive and irreversible condition of the arteries in which hyperplasia of one or more of the structural elements is a primary reaction, which results in thickening, dilation, deformity, and loss of elasticity of the wall of the artery). It is interesting to note that no atherosclerotic changes (i.e., arteriosclerosis characterized by lesions containing lipid deposits in and beneath the intima) were found in the carp even though they have been found in a few other species of teleosts (Vastesaeger et al., 1965). The carp lesions lack the intercellular fat deposits and foam cells (lipid-laden macrophages) indicative of atherosclerosis.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr Meyer Texon of the Department of Forensic Medicine, New York University School of Medicine, for his valuable comments concerning revision of the manuscript. This work was supported by a grant-in-aid of research from The Society of the Sigma Xi.

REFERENCES

CHU, Y. T. 1935. Comparative studies on the scales and on the pharyngeals and their teeth in Chinese cyprinids, with particular reference to taxonomy and evolution. Biol. Bull. St. John's Univ., Shanghai 2: 1–225.

COMFORT, A. 1964. Ageing: the biology of senescence. Routledge and Kegan Paul, London. 365 p.

LANKESTER, E. R. 1870. On comparative longevity in man and the lower animals. The Macmillan Co., London. 135 p.

TEXON, M. 1963. The role of vascular dynamics in the development of atherosclerosis, p. 167–195. *In* M. Sandler and G. H. Bourne [ed.] Atherosclerosis and its origin. Academic Press Inc., New York.

1967. Mechanical factors involved in atherosclerosis, p. 23–42. *In* A. N. Brest and J. H. Moyer [ed.] Atherosclerotic vascular disease. Appleton-Century-Crofts, New York.

VASTESAEGER, M., R. DELCOURT, AND P. GILLOT. 1965. Spontaneous atherosclerosis in fishes and reptiles, p. 129–149. *In* J. C. Roberts and R. Straus [ed.] Comparative atherosclerosis. Hoeber Med. Div. of Harper and Row, New York.

Geographic Variation in the Brook Stickleback, *Culaea inconstans*, and Notes on Nomenclature and Distribution

JOSEPH S. NELSON

Department of Zoology
The University of Alberta, Edmonton, Alta.

NELSON, JOSEPH S. 1969. Geographic variation in the brook stickleback, *Culaea inconstans*, and notes on nomenclature and distribution. J. Fish. Res. Bd. Canada 26: 2431–2447.

Clinal variation in dorsal and pelvic spine lengths was observed in the brook stickleback, *Culaea inconstans*, in an examination of 1366 specimens from 62 localities. Mean spine length was longest in the Wisconsin to Ohio area and generally decreased to the west, north, and east of this area. Shortest spines were observed in the northwestern part of the range of the species. A population in New Mexico, believed to be relict, was highly variable, but most specimens had long spines. Pelvic spines, and the supporting skeleton, were absent in most specimens from five localities in Alberta. Pelvic skeleton size and body depth were generally greatest in the area east of Wisconsin and least in the northwestern part of the range. Although *C. inconstans* is generally described as being naked, a series of about 30–36 small bony scutes was found along the lateral line in all populations examined. No marked geographic variation was observed in number of pectoral rays, caudal rays, soft dorsal-fin rays, soft anal-fin rays, gill rakers, scutes, or vertebrae. The nomenclature and distribution of *C. inconstans* are reviewed. The historical origin of clinal variation in spine length is discussed.

Received February 21, 1969

INTRODUCTION

THE BROOK STICKLEBACK, *Culaea inconstans* (Kirtland), occurs in streams, ponds, and lakes across North America from northeastern British Columbia and southwestern Northwest Territories to New Brunswick and from Nebraska to New York. In addition, several isolated populations exist outside this range.

Differing descriptions and figures of *Culaea* (a monotypic genus) in regional ichthyological works suggest considerable morphological variation in length of spines and in body depth in relation to body size. Several early workers (references in Eigenmann, 1887; Jordan and Evermann, 1896, p. 744; Bertin, 1925, p. 67, 165) recognize different subspecies or species on the basis of presumed morphological differences between populations. Eigenmann (1887) and Bertin (1925) are of the opinion that the intraspecific differences do not merit nomenclatorial recognition. However, a detailed analysis of geographic variation in the species has not heretofore been made.

This paper describes the morphology and geographic variation of several taxonomically important characters in *Culaea*. Behavioral studies by Hoogland

Printed in Canada (J1387)

et al. (1957), Hall (MS, 1956), and Reisman and Cade (1967), suggesting that spines are important as anti-predation devices and in intraspecific signalling systems in sticklebacks, prompted a consideration of the historical origin of geographic variation in spine length.

MATERIALS AND METHODS

The material studied comprised 1366 specimens from 62 localities (Table 1) throughout the range of the species. Body proportions were obtained, under magnification when desirable, with dial calipers graduated and read to 0.1 mm. Ratios were made by arithmetic division, rather

TABLE 1. Localities from which *C. inconstans* specimens were examined for Fig. 3. BC: University of British Columbia; OSM: Ohio State Museum; ROM: Royal Ontario Museum; UA: University of Alberta (UAD = uncatalogued material donated from various sources for study); UC: University of Connecticut; UNM: University of New Mexico; UM: University of Manitoba, uncatalogued; UMMZ: University of Michigan Museum of Zoology.

Locality	Approx. lat N long W	Museum no.	Standard length (*mm*)	Total no. specimens examined
Northwest Territories				
Mink Cr.	60°27′ 116°21′	BC61–337	21–42	7
British Columbia				
Fish Cr.	56 18 120 56	BC63–1244	45–55	30
Hiding Cr.	54 58 120 03	BC61–540	21–53	7
Alberta				
Sousa Cr. dr.	58 35 118 55	UAD	45–54	10
Cardinal L.	56 12 117 40	BC61–316	35–45	15
Summit L.	53 33 117 05	UAD	38–60	20
Carl Cr.	58 25 116 17	UAD	38–66	23
Kananaskis R. dr.	50 41 115 07	UA360	34–49	13
Clyde area	54 10 113 42	UAD	31–51	20
Little Fish L.	51 23 112 12	UA1612	27–46	10
Berry Cr. dr.	51 40 111 40	ROM6814	26–38	16
Saskatchewan				
Dickson Dam	50 15 109 24	ROM17815	24–43	14
Aitkow Cr.	51 10 106 40	ROM21421	34–48	20
Lacawana Cr.	50 30 104 40	UMMZ61555	23–56	16
Manitoba				
Sandy L.	50 31 100 00	BC59–511	31–48	20
La Salle R.	49 50 97 30	UMMZ180600	29–39	26
Rennie Cr.	49 50 95 40	UMMZ180601	33–47	27
Farnworth L. bog	58 42 94 03	UMSH–6	26–53	44
Ontario				
Thames R. dr.	42 30 82 10	UMMZ89021	20–35	32
Cedar Cr.	43 10 80 20	UMMZ89090	17–44	24
Murdock Cr.	48 05 80 05	UMMZ117686	26–56	27
Shelter Valley Cr.	43 57 78 02	UAD	28–43	9
Quebec				
Ile Bizard	45 30 73 54	ROM25445	25–41	18
Domaine Des Trois Pines	46 13 73 17	ROM25446	24–35	25
Rivière Ste. Anne	46 50 71 53	UAD	32–50	10
St. Gerard Magella area	46 49 71 26	UAD	37–50	10
New Brunswick				
Manners Sutton	45 30 67 05	ROM19626,19636	26–40	15
Montana				
Redwater R.	47 20 105 45	UAD	29–43	26
Snake Cr.	48 20 105 00	UMMZ173939	20–48	12

(*Continued*)

TABLE 1. Localities from which *C. inconstans* specimens were examined for Fig. 3. BC: University of British Columbia; OSM: Ohio State Museum; ROM: Royal Ontario Museum; UA: University of Alberta (UAD = uncatalogued material donated from various sources for study); UC: University of Connecticut; UNM: University of New Mexico; UM: University of Manitoba, uncatalogued; UMMZ: University of Michigan Museum of Zoology. — (*Concluded*)

Locality	Approx. lat N long W	Museum no.	Standard length (*mm*)	Total no. specimens examined
North Dakota				
L. Souris Refuge Dam	48°40′ 100°50′	UMMZ114445	39–49	11
Apple Cr.	46 45 100 40	UMMZ94827	35–50	24
Devils L.	48 05 98 50	UAD	39–48	20
Wild Rice R.	46 30 96 50	UAD	30–39	17
South Dakota				
Sixmile Cr.	44 20 96 30	UMMZ166873	21–54	50
Nebraska				
Clear Cr.	41 10 96 20	UAD	31–38	15
Missouri R.	41 20 95 50	UMMZ162263	44–52	7
New Mexico				
Merrick L.	36 55 105 00	UNM602,603	25–54	39
Minnesota				
Buffalo R. dr.	46 50 96 20	UAD	27–45	17
Belle Cr.	44 25 92 45	UMMZ72386	36–45	22
Iowa				
Mud L.	43 20 94 40	UMMZ146753	23–39	34
Willow Cr.	43 10 93 20	UMMZ100988	38–52	21
Ed. Dalton State L.	42 10 90 30	UMMZ100953	22–41	27
Wisconsin				
Sand L.	45 51 91 30	UAD	30–47	32
Sioux R.	46 43 90 54	UAD	30–48	25
Big Spring Cr.	43 40 89 40	UMMZ73497	28–47	30
Black Cr.	45 10 89 10	UMMZ74387	22–53	23
Grand R. dr.	43 45 88 55	UMMZ73834	27–41	21
Illinois				
Rock R.	42 15 89 10	UAD	22–43	25
Michigan				
Minau R. dr.	46 20 86 40	UMMZ84338	21–57	52
Thread Cr.	42 55 83 40	UMMZ82164	18–31	28
Clinton R. dr.	42 35 83 05	UMMZ106955,107015	16–46	17
Indiana				
Crooked L.	41 16 85 29	UAD	15–34	27
Pretty L.	41 38 85 16	UAD	25–32	5
Ohio				
Macochee Cr.	40 10 83 50	OSM11-325	26–45	32
Miller Blue Hole Cr.	41 25 83 10	OSM5704	20–38	21
Cold Cr.	41 25 82 30	OSM2853	15–24	16
Tippecanoe Cr.	41 00 80 40	OSM4939	43–48	11
Shenango R.	41 10 80 40	OSM10000	30–40	32
New York				
Cayuga inlet	42 25 76 31	UAD	24–36	36
Onondaga Cr.	43 00 76 12	UAD	26–30	26
N. Wingfield Cr.	42 50 75 15	UMMZ109772	27–37	28
Connecticut				
Dry Brook	41 51 72 35	UC2233	22–50	29

than by stepping the length of the part into the base length. Stained and cleared specimens were prepared according to the enzyme digestion technique of Taylor (1967). Drawings were made with the aid of a camera lucida.

The following characters were examined. *Body depth* was measured at the deepest part of the body. *Pelvic spine length* was measured on the left side from the external base to the tip. Specimens with markedly unequal right and left spines were not included. Length was measured with the spines in a half-erected state. *First dorsal spine length* was measured from the origin to the tip. Length was measured with the spines in a half-erected state. *Dorsal spine number* included all spine elements. *Numbers of pectoral, soft anal,* and *soft dorsal rays* were determined on stained specimens and included all soft ray elements. *Caudal ray number* was the count of principal rays, generally the number of branched rays plus two. Counts were made on stained specimens. *Gill rakers* were counted on the left first arch on stained and cleared specimens. Every bony rudiment was included. *Vertebrae* were counted in stained and cleared specimens. The first post-caudal vertebrae supported the heavy haemal spine leading to the anal spine. Many specimens from Cayuga Lake and Nebraska had deformed columns and these specimens were not employed. *Scutes* were counted on the left side on stained specimens. This count did not include the one to six scutes which are paired with the anterior-most scutes and are out of linear series (Fig. 1).

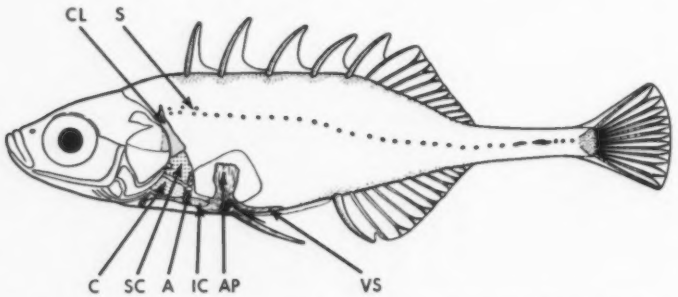

FIG. 1. Stained *C. inconstans* (21.1 mm standard length) from Crooked Lake, Indiana, showing scutes and parts of the pectoral and pelvic skeletons. A = actinosts supporting pectoral fin; AP = ascending branch of pelvic skeleton; C = coracoid; CL = cleithrum; IC = interclavicle process of the coracoid; S = scutes; SC = scapula; VS = ventral shield of pelvic skeleton.

The relations of dorsal and pelvic spine lengths to standard length were graphed by plotting the original variates for each sample; pelvic spine length is plotted against standard length for five populations in Fig. 2. Generally, ranks were assigned to each plot by determining the intercept at 32.5 mm standard length from eye-fitted regression lines. Extrapolations were made, when necessary, by employing samples of large range in size and exhibiting several degrees of spine length. Nine ranks were recognized (Table 2). Samples with the shortest mean spine length were ranked lowest and plotted as open circles (Fig. 3). Those with the longest mean spine length were ranked highest and plotted as solid circles. The degree of difference in other samples was shown in proportion. Some samples had irregular lines of fit. In these instances, the line which best seemed to approximate the typical curve was employed.

In Fig. 3, numerous regional works were employed for distributional limits within or near the contiguous range. Records from James Bay and central Quebec are from Cox (1921) and Richardson (1944). It is not known if the disjunct population examined from Connecticut is native or introduced.

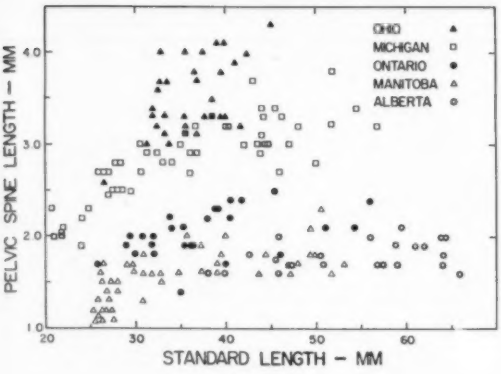

FIG. 2. Differences among five populations of *C. inconstans* in the relation of pelvic spine length to standard length. Ohio: Champaign County, Macochee Creek; Michigan: Alger County, Minau drainage; Ontario: Murdock Creek; Manitoba: bog near Farnworth Lake; Alberta: Carl Creek.

VARIATION IN SPINE LENGTH

Specimens from Farnworth Lake bog in Manitoba, Rock River in Illinois, and Macochee Creek and Shenango River in Ohio were examined to determine if dorsal or pelvic spine length varied with sex. No such variation was apparent.

The regressions of dorsal and pelvic spine lengths against standard length showed much variation throughout the range of *Culaea* (e.g., Fig. 2).

The ranking of plots from the 62 populations showed that spines were generally longest in the Wisconsin to Ohio area and shortest in the northwestern part of the range (Fig. 3). Spine length tended to decrease in populations to the west, north, and east of the mid-west area. Marked differences were occasionally apparent in relatively close populations. For example, the St. Gerard Magella, Quebec, population had longer spines than its neighbors, whereas the Cayuga, New York, population had shorter dorsal spines. These regional differences, however, did not override the general trend of clinal variation. In some instances the rankings would have been different if the chosen intercept (32.5 mm) had been different but the general pattern would remain unchanged.

The New Mexico sample, caught in 1961, showed exceptionally large variation in dorsal and pelvic spine lengths. Individuals between 33 and 35 mm standard length had pelvic spines varying from 2.5 to 3.8 mm in length. The long spines (mean ranked as 3/4) were associated with pale colored individuals with, generally, a typical mouth shape, whereas the short spines (mean ranked as 3/8) were associated with the relatively few blackish individuals, which generally had a deformed mouth. Unfortunately sex could not be determined, although males appeared to be present in both groups. Interestingly, of four specimens caught in 1954, the two lightest colored ones had the greater relative spine lengths whereas the two darkest had the lesser relative spine lengths. All appeared to be males and had normal mouths.

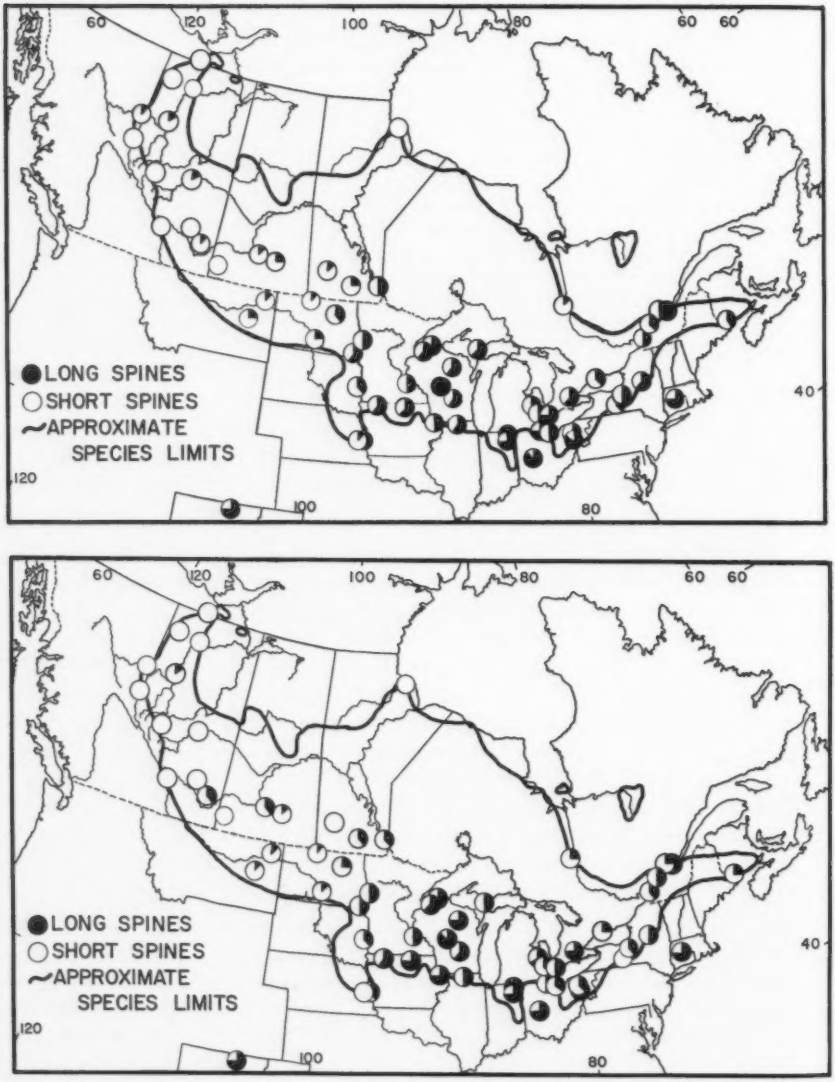

FIG. 3. Variation in lengths of pelvic (*upper*) and first dorsal (*lower*) spines. Open circles denote populations with shortest mean spine length; solid circles, those with longest mean spine length. Ratio of white to black in other populations is proportional to mean spine length. See text and Table 2 for further explanation.

TABLE 2. Ranks assigned to various spine lengths of *C. inconstans* for Fig. 3. Mean spine length was determined at 32.5 mm standard length. See text for further explanation.

Rank	Mean length (*mm*)	
	First dorsal	Pelvic
0	1.17 or less	1.83 or less
1/8	1.18–1.32	1.84–2.11
1/4	1.33–1.47	2.12–2.38
3/8	1.48–1.62	2.39–2.66
1/2	1.63–1.77	2.67–2.93
5/8	1.78–1.92	2.94–3.21
3/4	1.93–2.07	3.22–3.48
7/8	2.08–2.22	3.49–3.76
1	2.23 or more	3.77 or more

Correlation coefficients between spine length and standard length over the size range shown in Fig. 2 were as follows:

	r value		t value		P		df	
Population	Dorsal	Pelvic	Dorsal	Pelvic	Dorsal	Pelvic	Dorsal	Pelvic
Minau Creek, Mich.	0.35	0.55	13.0	25.7	<0.001	<0.001	33	33
Murdock Creek, Ont.	0.29	0.51	7.8	17.4	<0.001	<0.001	25	25
Farnworth Lake bog, Man.	0.47	0.04	25.5	1.5	<0.001	>0.10	42	42
Carl Creek, Alta	0.22	0.44	4.8	11.3	<0.001	<0.001	21	21

Some populations showed a negative correlation between spine and body lengths in the larger individuals (e.g., Willow Creek, Iowa, $r = -0.63$ ($t = 19.8$, $P < 0.001$, df = 19) for dorsal spines and $r = -0.69$ ($t = 25.0$, $P < 0.001$, df = 19) for pelvic spines; Aitkow Creek, Saskatchewan, $r = -0.54$ ($t = 12.2$, $P < 0.001$, df = 18) for dorsal spines). Several other populations showed no obvious change in spine length with increase in body size in adults.

The correlation between mean dorsal and pelvic spine lengths in the 62 populations was high. Spearman's coefficient of rank correlation (Simpson et al., 1960), appropriate for ranked characters, was 0.91. Graphical analyses of the relationship of dorsal to pelvic spine length showed the positive relationships to be weaker within than between populations; considerable dispersion occurred. Correlation coefficients were calculated for the four populations in Fig. 2 which had a large size range: Minau Creek, Michigan, $r = 0.71$ ($t = 47.5$, $P < 0.001$, df = 33); Murdock Creek, Ontario, $r = 0.34$ ($t = 9.8$, $P < 0.001$, df = 25); Farnworth Lake bog, Manitoba, $r = 0.70$ ($t = 57$, $P < 0.001$, df = 42); Carl Creek, Alberta, $r = 0.45$ ($t = 12$, $P < 0.001$, df = 21).

McPhail (1963) found variation in pelvic spine length in *Pungitius pungitius* but clinal variation was not observed within the form occupying much of the area occupied by *Culaea*.

VARIATION IN SHAPE AND SIZE OF PELVIC AND PECTORAL SKELETONS

Marked differences in the shape and size of the pelvic skeleton in relation to that of the body were noted. Generally, the pelvic skeleton was largest in proportion to body size in the Wisconsin to New York area and least developed in the northwestern part of the range (Fig. 4). The mid-west populations

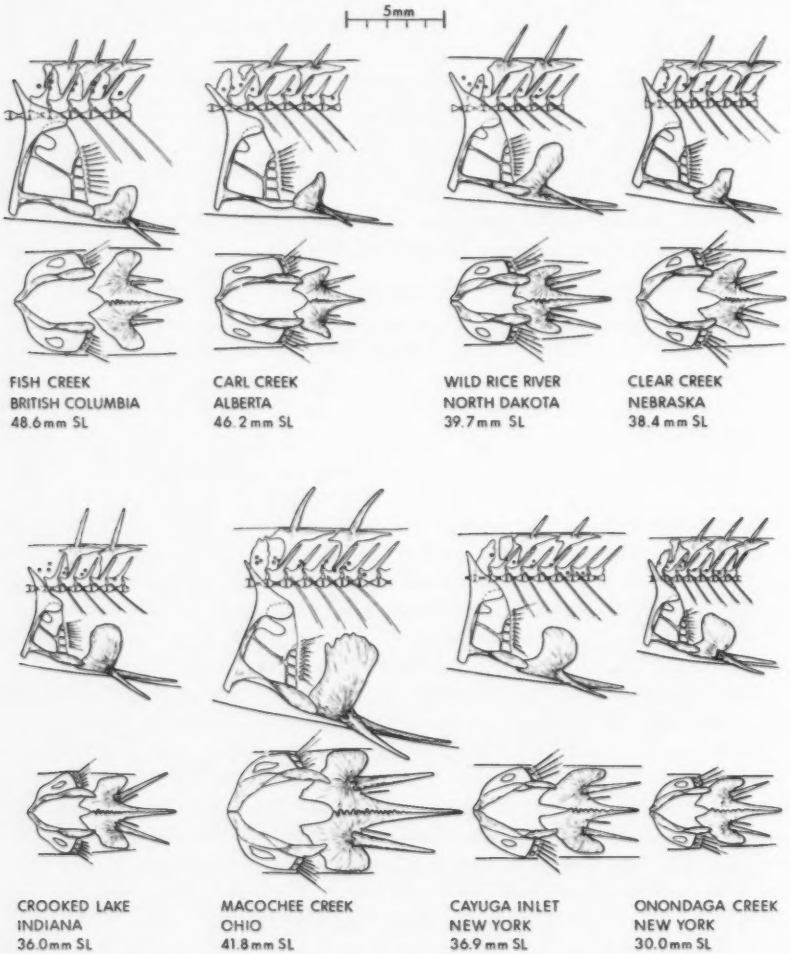

FIG. 4. Stained and cleared *C. inconstans* to show variations in shape and size of pelvic skeleton and length of dorsal and pelvic spines. Specimens are representative of their populations but do not necessarily represent the mean spine length for their populations.

typically had a broad lateral ascending process, usually extending more than one-half the way to the vertebral column on the relatively deep body. The ventral shield was elongate. Populations from the northern and western parts of the range had a much reduced pelvic skeleton. The lateral ascending process usually tapered to an end less than one-half way up to the vertebral column on the relatively shallow body. The ventral shield was short. A positive relationship occurred for the ratio of size of the pelvic skeleton to length of the pelvic spine between populations.

Five populations were found in Alberta in which the pelvic skeleton was absent (determined by external examination with a needle under magnification and verified by staining in many specimens) in most of the individuals (Table 3). Populations having the pelvic skeleton occurred around and within the

TABLE 3. Populations of *C. inconstans* in Alberta in which most individuals lack the pelvic skeleton and spines. Specimens are from the University of Alberta and the Alberta Fish and Wildlife Division.

Locality	Approx lat N long W	No. with: No pelvic skeleton	Part of skeleton	Full skeleton
Corbett Cr.	54°17′ 115°14′	11	0	0
Muir L. 1960	53 37 113 57	65	1	22
1968		101	6	38
Astotin L.	53 42 112 52	836	10	655
Trib. to Punk Cr.	54 32 111 14	13	10	2
Cold L.	54 28 110 12	9	1	5

broad area of these five populations. The only specimen which has been examined from Pine Lake, Alberta, 59°34′, 112°14′ (National Museum of Natural Sciences, NMC 67-19), also lacked the pelvic skeleton. A higher proportion of young specimens (11–30 mm standard length) than adults (52–71 mm standard length) lacked the pelvic skeleton in the large Astotin Lake sample, collected in 1968. Also, most young specimens had six dorsal spines whereas most adults had five dorsal spines. No simple relationship between the number of dorsal spines and the presence or absence of the pelvic skeleton was apparent within size groups. Outside of a few morphological intermediates in four of the five populations, the variations in spine length and pelvic skeleton shape were within the ranges of variation for other Alberta populations. No gradation existed between the spined and spineless individuals. A similar situation has been reported in *Pungitius*; Day (1878) noted 11 out of 13 specimens from inland waters of Ireland to be lacking in pelvic spines and skeleton.

The general shape of the pelvic skeleton in *Culaea* is similar to that of *Pungitius* and *Gasterosteus*. The most noticeable difference is the lack of lateral

ribs or struts in the ventral shield of *Culaea*, which are usually present in *Pungitius* and *Gasterosteus*. Marked differences in shape and size of the pelvic skeleton, although not of a clinal nature, have been observed in other sticklebacks (*Pungitius* — Nelson, 1968a; *Gasterosteus* — Day, 1878; Penczak, 1960, 1962, 1965). Penczak (1965) found some differences to be associated with sex and felt there was a tendency for the skeleton to be reduced in increasing salinities.

No clinal variation was observed in the pectoral skeleton. The interclavicle process (infracleithrum of Swinnerton, 1905 and Banister, MS, 1967), contrary to the statement of Starks (1903), develops as a cartilage bone separately from the dermal coracoid (hypocoracoid of Starks) of the pectoral skeleton (Swinnerton, 1905). In *Culaea*, the interclavicles are short, lacking the forward extension to the midline at the junction of the cleithra (clavicle of Starks, clavicule of Bertin, 1925) characteristic of *Gasterosteus*, *Pungitius*, and *Spinachia*. Their shape in *Culaea* as figured and described by Hall (MS, 1956, fig. 25, p. 343) was never observed in the present study. The interclavicles in *Culaea* and *Apeltes* are much more similar than Hall suggests. The main difference is the relative forward position of the small bones, together with the pelvic skeleton, in *Apeltes*. Leiner (1934, fig. 12, p. 130) also appears to be in error concerning the shape of the interclavicles in *Culaea* and was incorrectly noted by Hall to have stated that their shape is similar to that in *Apeltes* (which it is, however). This error possibly resulted from Hall's interpreting Leiner's dwarf stickleback as *Apeltes* rather than *Pungitius*. Leiner correctly described the interclavicles in *Apeltes* but incorrectly felt that *Culaea* resembled *Pungitius*.

VARIATION IN BODY DEPTH

The relation between body depth and size showed much variation throughout the range of the species (Fig. 5). Samples are arranged in longitudinal sequence in Canada and the United States, respectively. The pattern was more

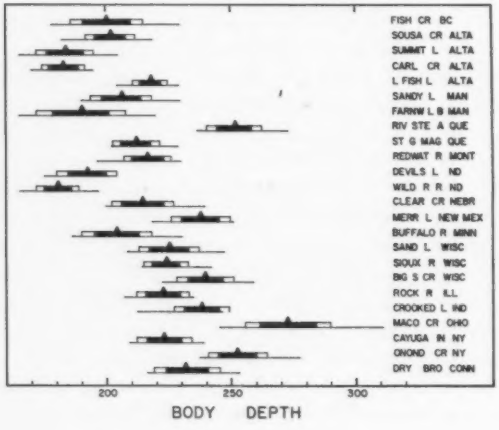

FIG. 5. Variation in body depth (thousandths of standard length) in *C. inconstans*. Each population is represented by 10 specimens (total range 25–54 mm standard length). Black bar, four standard errors of the mean; black bar plus white bar on either side of the mean, one standard deviation; horizontal line, range.

irregular than for spine length (perhaps partly based on seasonal and sex differences in body depth). Spearman's coefficient of rank correlation for pelvic spine length and body depth was 0.63 and for dorsal spine length and body depth it was 0.60 for the 24 populations studied. The coefficient of correlation for body depth and spine length within populations was highly variable. For example, in Farnworth Lake bog, Manitoba, $r = -0.03$ ($t = 0.2$, $P > 0.50$, df = 8) for dorsal spines and $r = -0.40$ ($t = 3.8$, $P < 0.01$; df = 8) for pelvic spines; in Gerard Magella, Quebec, $r = -0.31$ ($t = 2.7$, $P < 0.05$, df = 8) for dorsal spines and $r = -0.30$ ($t = 2.6$, $P < 0.05$, df = 8) for pelvic spines; and in Rivière Ste. Anne, Quebec, $r = 0.26$ ($t = 2.2$, $P > 0.05$, df = 8) for dorsal spines and $r = 0.77$ ($t = 14.9$, $P < 0.001$, df = 8) for pelvic spines.

Culaea in the southwestern part of its range is a relatively deep-bodied, compressed fish, much different from *Pungitius*. For example, Nelson (1968b) found a distinct gap in the body depth relationship between sympatric *Culaea* and *Pungitius* in Indiana. In the northwestern part of its range *Culaea* is slender and similar to *Pungitius*.

VARIATION IN MERISTIC CHARACTERS

The numbers of fin rays (spines and soft rays), gill rakers, scutes, and vertebrae in populations from most of the range of the species (Table 4) showed little geographic variation and a detailed analysis was therefore not made.

TABLE 4. Summary of meristic data for characters not showing marked clinal variation. Populations from throughout much of the range of the species.

Character	Mean	Range	No. of populations	No. of specimens
Pectoral rays	9.9	9–11	14	80
Dorsal soft rays	9.8	8–13	14	85
Anal soft rays	9.9	7–12	14	85
Caudal rays	11.9	11–12	14	85
Gill rakers				
Upper arch	3.5	2–5	9	49
Lower arch	8.7	7–10	9	49
Total	12.3	10–15	15	164
Lateral scutes	32.9	30–36	14	80
Vertebrae				
Precaudal	13.8	13–15	13	77
Postcaudal	18.1	17–20	13	77
Total	32.0	30–35	13	77

Hansen's (1939) study of Illinois *Culaea* and unpublished data from Schmid's (1963) study of North Dakota *Culaea* showed that the modal number of rays was 10 for the pectoral, dorsal, and anal fins (most counts were between 9 and 11) and was 12 for the caudal rays. This was also found in the present study, with one exception, in populations from British Columbia to New

York. The one exception was found in Crooked Lake, Indiana, where most specimens had 9 pectoral rays (range 8–10). Unpublished data from Schmid's (1963) study suggested little asymmetric variation in number of pectoral fin rays and little sexual variation in the number of dorsal spines and pectoral, dorsal, anal, and caudal soft rays.

The modal count of dorsal spines for most populations was five. The vast majority of individuals had four to six spines with a total variation of four to seven (as was found by Lawler, 1958). Hansen (1939) found two to seven spines in samples of 10 populations from Illinois totaling about 2200 fish; the modal count was five and the range for 98% of the individuals was four to six. Cahn (1927) and Schmid (1963; unpublished data) found four to six (majority with five) spines in 100 specimens from Wisconsin and 291 specimens from North Dakota, respectively. Lawler (1958) presented evidence of a north–south cline in spine number in Manitoba and Ontario. No definite cline was noted in the present study; however, counts of six predominated in the high-altitude New Mexico population and in some British Columbia and Alberta populations.

Comparative data from other studies on the number of gill rakers in *Culaea* could not be found. Their number (10–15) is relatively low and comparable with counts in *Pungitius* (McPhail, 1963).

Although *Culaea* is generally described as lacking scutes, staining with Alizarin Red S clearly shows a row of 30–36 small, nonoverlapping lateral scutes (Fig. 1 and 4), as first noted in an earlier study (Nelson, 1968b). Most of the scutes have a central pore. Scutes present on the caudal peduncle resemble developing scutes on young *Pungitius* (as observed on an Indiana population) and sometimes become fused. Scutes, in general, resembled some of the isolated elements on the anterior portion of the lateral line in *Gasterosteus* and *Pungitius* as described by Igarashi (1962, 1963, 1964, 1965). In Crooked Lake, Indiana, most specimens over 21 mm standard length appeared to have the mean adult complement of scutes. They appeared to be completely laid down only in larger individuals in several other populations. Although the range for the number of scutes about equaled the range for the number of vertebrae, there was not a marked 1:1 relationship noted within the populations. Median dorsal scutes behind the dorsal fin are very underdeveloped in *Culaea* compared with those of other species. Of the seven genera of Aulorhynchidae and Gasterosteidae, only the fourspine stickleback, *Apeltes*, is now known to completely lack lateral scutes.

NOMENCLATURE AND DISTRIBUTION

The first mention of the brook stickleback was made by Kirtland (1838), as "*G.*" *inconstans*, and the first adequate description was made by Kirtland (1841), as *Gasterosteus inconstans*, the variable stickleback, based on specimens from Trumbull County, Ohio. Various workers after Kirtland recognized different species or subspecies; however, Eigenmann (1887) and Bertin (1925)

were of the opinion that the intraspecific differences did not merit nomenclatorial recognition. The present study, in showing clinal variation, supports their contention. Dawson's (1859) study of *Gasterosteus gymnetes* from Montreal, an excellent early description of the brook stickleback, has been missed in synonomies of the species (Jordan and Evermann, 1896; Jordan et al., 1930). Jordan (1876) placed the brook stickleback in the genus *Eucalia* and characterized the new group in 1877. Whitley (1950, p. 44) listed *Eucalia* as being preoccupied (by a nymphalid butterfly; *Eucalia*, applied in 1861, is a junior synonym of *Hypolimnas*) and proposed the name *Culaea*. The latter name has been accepted in the literature since it was brought to the attention of North American workers by Bailey and Allum (1962).

Culaea is often very common in the fish fauna throughout much of its range. Populations occur in such diverse environments as high altitude streams and lakes (1668 m in Alberta — Nelson, 1965; 2485 m in New Mexico — Koster, MS, 1968), brackish water (in James Bay — Cox, 1921), saline prairie lakes (Hankinson, 1929; Rawson and Moore, 1944), and an artesian well in South Dakota (Bean, 1903). Usually, in all environments, they are associated with aquatic vegetation (Whitaker, MS, 1968). Although their tolerance to salt water is the lowest of all sticklebacks for which comparable data are available, brook sticklebacks appear to have a greater salinity tolerance than other North American fish found only in fresh water (Nelson, 1968c).

The approximate range limits of *Culaea* are shown in Fig. 3 (collections from northeastern New Brunswick and southwestern Quebec are not shown). Koster (1957) reported a population of *Culaea* from Merrick Lake, upper Canadian Basin, northeastern New Mexico. This population, found in 1954, is known to be still extant despite an eradication program on the lake in 1954 and Koster (MS, 1968) presents convincing evidence favoring the belief that the population is native. *Culaea* has also been taken in the Stubblefield Reservoir area and in some minnow ponds in New Mexico. Koster believes the former could have originated from Merrick Lake but the latter seem to have been introduced from Minnesota (*Culaea* is commonly found with *Pimephales promelas* in bait shipments from Minnesota and Wisconsin in other states as well — indeed, Smith-Vaniz (1968) reports its collection from as far south as northwestern Alabama near a minnow hatchery containing *Culaea*). Early workers gave the range limits of *Culaea* as in Kansas and Greenland (Jordan and Gilbert, 1883; Eigenmann, 1887). Both extremes are doubtful. Cope's (1865) record from Greenland needs verification and his report of it in Kansas may be in error (Cross, 1967). Recently, McPhail and Lindsey (1969) have extended the known northward range of the species by reporting three populations in the Northwest Territories, south of Great Slave Lake.

GENERAL DISCUSSION

The historical origin of clinal variation in spine length, pelvic skeleton shape, and body depth in *Culaea* is open to speculation. The differences, if they have a genetic basis, could have had several possible origins (considerable

environmental influence may be involved — for example, the shorter spined populations tend to be in regions with cooler temperatures).

Clinal patterns may result if differentiation in separate refugia is followed by secondary intergradation. Many species have had separate refugia during the Wisconsin glaciation (e.g., Missouri–Mississippi areas, Yukon–Mississippi areas, and Columbia–Mississippi areas). If *Culaea* did have a double refugia, it would have been a Missouri–Mississippi one. The upper Missouri basin is believed to have been a refugium for some fish (Bailey and Allum, 1962). The occurrence of *Culaea* in the western Alberta area makes a Missouri refugium seem plausible. The species' absence from the Wyoming and eastern South Dakota area, although not ruling out a double refugia, does tend to support a sole Mississippi refugium hypothesis. It is certainly possible, however, that the species did occur in separate refugia during glaciation, with the extreme differences arising in allopatric areas, and the clinal pattern resulting from gene flow after re-entry into the glaciated areas.

Another possibility is that the populations are under a common selective pressure of varying strength that results in unequal rates of change or evolution. This view, that predation, for example, is less intense in the northwestern area than in the mid-west, although possibly true, marshals little support with current information.

The hypothesis that the characters of each population, in general, are local adaptations to a clinal environment seems plausible with the available evidence. Hoogland et al. (1957) found that de-spined (short-spined) *Gasterosteus* were more vulnerable to predation than the controls (with long spines) and that *Pungitius* (short-spined) was taken by the predators sooner than *Gasterosteus* (long-spined). Although emphasis in this experimental work was on the importance of long spines, the authors provided data which may suggest why many *Culaea* populations have short spines. Hoogland et al. (1957) found that avoidance of sticklebacks by the predators occurred after experience with them, but only in choice situations. In the northwestern part of its range, where *Culaea* has short spines, the ratio of forage fish to predators is relatively low compared with the mid-west, where *Culaea* has long spines. Consequently, predators in the northwestern part of the range have less choice than predators in the mid-west and, under these conditions, natural selection may favor escape responses (i.e., favor short-spined individuals with a streamlined body and light pelvic skeleton and better able to escape into dense vegetation).

The clinal variation in spine length also suggests that geographic differences may exist not only in susceptability to predation in similar environments but also in intraspecific behavior. Hall (MS, 1956), in a comparative study of stickleback behavior, concluded that spines in *Culaea* were important as intraspecific signalling devices, both in agonistic encounters and in sexual behavior (Reisman and Cade, 1967, observed much the same; however, as noted by McKenzie (MS, 1964), the significance of spine erection requires further study). Although young-of-the-year under natural conditions do form schools

in the fall and spring, adults are highly territorial (Hall, MS, 1956; McKenzie, MS, 1964). Hall found that agonistic behavior appeared to be more developed in *Culaea* than in other stickleback species.

The general importance of spines in *Culaea* might also be indicated by the presence of melanophores on the interradial membranes of the dorsal and anal fins and on the dorsal spine membranes. This pigment, which makes the spines conspicuous, is usually reduced or absent on other North American sticklebacks, *Gasterosteus* and *Pungitius*, and is abundant in *Apeltes* only on the dorsal spine membrane.

ACKNOWLEDGMENTS

I express my thanks to Dr G. E. Ball, Dr J. R. Nursall, and Mr G. E. E. Moodie for critically reading the manuscript.

I am indebted to many individuals for material. The following persons donated specimens for study: M. J. Paetz, R. J. Paterson, J. C. Ward (Alberta), E. D. Lane (Ontario), J. Bergeron (Quebec), J. B. Owen, J. J. Peterka (North Dakota), L. Morris (Nebraska), T. D. Beard (Wisconsin), P. W. Smith (Illinois), R. E. Jenkins and R. G. Werner (New York). Loans of material were made from J. D. McPhail, P. J. Ellickson (The University of British Columbia), C. C. Lindsey (The University of Manitoba), W. B. Scott, E. J. Crossman (Royal Ontario Museum), D. E. McAllister (National Museum of Natural Sciences), W. J. Koster (The University of New Mexico), R. M. Bailey (University of Michigan), M. B. Trautman (Ohio State Museum), and R. M. Wetzel (The University of Connecticut).

Information on the distribution of *Culaea* was kindly provided by F. M. Atton (Saskatchewan Fisheries Branch), C. C. Lindsey (The University of Manitoba), and D. E. McAllister (National Museum of Natural Sciences). I am grateful to J. D. McPhail, R. M. Bailey, and M. B. Trautman for help and encouragement in various ways, and to W. J. Koster and W. D. Schmid for supplying much valuable unpublished material. Permission to collect in Astotin Lake, Elk Island National Park, was kindly given by the Department of Indian Affairs and Northern Development and Park Superintendent T. L. Ross.

The study was started at Indiana University under the sponsorship of the Indiana Aquatic Research Unit. Financial support from the National Research Council of Canada, grant number A-5457, permitted the completion of the study.

REFERENCES

BAILEY, R. M., AND M. O. ALLUM. 1962. Fishes of South Dakota. Misc. Publ. 119, Museum Zool., Univ. Mich. 131 p.

BANISTER, K. E. MS, 1967. The anatomy and classification of the order Gasterosteiformes (Pisces). Ph.D. Thesis. University of Newcastle upon Tyne, England. 211 p.

BEAN, T. H. 1903. Catalogue of the fishes of New York. New York State Museum, Bull. 60. 784 p.

BERTIN, L. 1925. Recherches bionomiques, biométriques et systématiques sur les Épinoches (Gastérostéidés). Ann. Inst. Oceanog. Monaco 2: 1–204.

CAHN, A. R. 1927. An ecological study of southern Wisconsin fishes. Illinois Biol. Monographs 11: 1–151.

COPE, E. D. 1865. Partial catalogue of the cold-blooded Vertebrata of Michigan. Pt. 2. Proc. Acad. Nat. Sci. Phila. 17: 78–88.

COX, P. 1921. Results of the Hudson Bay Expedition, 1920. II. The Gasterosteidae. Contrib. Canadian Biol. 1921: 151–153.

CROSS, F. B. 1967. Handbook of fishes of Kansas. Misc. Publ. 45, Museum Nat. Hist., Univ. Kansas. 357 p.

DAWSON, J. W. 1859. On a new species of stickleback (*Gasterosteus gymnetes*). Canadian Naturalist Geologist 4: 321–324.

DAY, F. 1878. On some Irish Gasterostei. J. Linnean Soc. London (Zool.) 13: 110–114.

EIGENMANN, C. H. 1887. A review of the American Gasterosteidae. Proc. Acad. Nat. Sci. Phila. 1886: 233–252.

HALL, M. F. MS, 1956. A comparative study of the reproductive behaviour of the sticklebacks (Gasterosteidae). Ph.D. Thesis. St. Hugh's College, Oxford. 370 p.

HANKINSON, T. L. 1929. Fishes of North Dakota. Papers Mich. Acad. Sci. 10: 439–460.

HANSEN, D. F. 1939. Variation in the number of spines and rays in the fins of the brook stickleback. Trans. Illinois Acad. Sci. 32: 207–212.

HOOGLAND, R., D. MORRIS, AND N. TINBERGEN. 1957. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (*Perca* and *Esox*). Behaviour 10: 205–236.

IGARAS[H]I, K. 1962. Observations on the development of the scutes in ten-spined stic[k]leback, *Pungitius sinensis* (Guichenot). Bull. Japan. Soc. Sci. Fish. 28: 393–398

1963. Observation on the development of the scutes in ten-spined stic[k]leback, *Pungitius pungitius* (Linnaeus). Bull. Japan. Soc. Sci. Fish. 29: 342–348.

1964. Observation on the development of the scutes in landlocked form of three-spined stic[k]leback, *Gasterosteus aculeatus aculeatus* Linnaeus. Bull. Japan. Soc. Sci. Fish. 30: 95–103.

1965. Observation on the development of the scute in landlocked form of three-spined stickleback, *Gasterosteus aculeatus microcephalus* Linnaeus. Bull. Japan. Soc. Sci. Fish. 31: 33–40.

JORDAN, D. S. 1876. Manual of the vertebrates of the northern United States. 1st ed. Jansen, McClurg and Company, Chicago, Ill. 342 p.

1877. On the fishes of northern Indiana. Proc. Acad. Nat. Sci. Phila. 1877: 42–82.

JORDAN, D. S., AND B. W. EVERMANN. 1896. The fishes of North and middle America. Bull. U.S. Natl. Museum 47: 1240 p.

JORDAN, D. S., B. W. EVERMANN, and H. W. CLARK. 1930. Check list of the fishes and fishlike vertebrates of North and middle America. Rept. U.S. Comm. Fish. 1928, App. 10. 670 p.

JORDAN, D. S., AND C. H. GILBERT. 1883. Synopsis of the fishes of North America. Bull. U.S. Natl. Museum 16: 1018 p.

KIRTLAND, J. P. 1838. A catalogue of the Mammalia, birds, reptiles, fishes, Testacea, and Crustacea in Ohio. Ann. Rept. Geol. Surv. Ohio 2: 157–197.

1841. Descriptions of four new species of fishes. Boston J. Nat. Hist. 3: 273–277.

KOSTER, W. J. 1957. Guide to the fishes of New Mexico. University of New Mexico Press, Albuquerque, New Mexico. 116 p.

MS, 1968. The origin of the *Semotilus*, *Chrosomus*, and *Culaea* populations in New Mexico. 19 p.

LAWLER, G. H. 1958. Variation in number of dorsal spines in the brook stickleback, *Eucalia inconstans*. Canadian J. Zool. 36: 127–129.

LEINER, M. 1934. Die drei Europäischen stichlinge (*Gasterosteus aculeatus* L., *Gasterosteus pungitius* L. und *Gasterosteus spinachia* L.) und ihre kreuzungsprodukte. Z. Morphol. Ökol. Tiere 28: 107–154.

MCKENZIE, J. A. MS, 1964. The reproductive behavior of the brook-stickleback *Eucalia inconstans* (Kirtland). Ph.D. Thesis. University of Western Ontario, London, Ont. 135 p.

MCPHAIL, J. D. 1963. Geographic variation in North American ninespine sticklebacks, *Pungitius pungitius*. J. Fish. Res. Bd. Canada 20: 27–44.

MCPHAIL, J. D., AND C. C. LINDSEY. 1969. Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Bd. Canada. (In press.)

NELSON, J. S. 1965. Effects of fish introductions and hydroelectric development on fishes in the Kananaskis River system, Alberta. J. Fish. Res. Bd. Canada 22: 721–753.

1968a. Deep-water ninespine sticklebacks, *Pungitius pungitius*, in the Mississippi drainage, Crooked Lake, Indiana. Copeia 1968: 326–334.

1968b. Ecology of the southernmost sympatric population of the brook stickleback, *Culaea inconstans*, and the ninespine stickleback, *Pungitius pungitius*, in Crooked Lake, Indiana. Indiana Acad. Sci. 77: 185–192.

1968c. Salinity tolerance of brook sticklebacks, *Culaea inconstans*, freshwater ninespine sticklebacks *Pungitius pungitius*, and freshwater fourspine sticklebacks, *Apeltes quadracus*. Canadian J. Zool. 46: 663–667.

PENCZAK, T. 1960. New cases of variability of the stickleback *Gasterosteus aculeatus* L. Fragmenta Faunistica, Warszawa 8: 403–410.

1962. Morphometry of the stickleback, *Gasterosteus aculeatus* L., from the Baltic Sea. Zool. Polon. 12: 239–246.

1965. Morphological variation of the stickleback (*Gasterosteus aculeatus* L.) in Poland. Zool. Polon. 15: 3–49.

RAWSON, D. S., AND J. E. MOORE. 1944. The saline lakes of Saskatchewan. Canadian J. Res. 22(D): 141–201.

REISMAN, H. M., AND T. J. CADE. 1967. Physiological and behavioral aspects of reproduction in the brook stickleback, *Culaea inconstans*. Am. Midland Naturalist 77: 275–295.

RICHARDSON, L. R. 1944. Brief record of fishes from central northern Quebec. Copeia 1944: 205–208.

SCHMID, W. D. 1963. Covariation of some meristic characters of the brook stickleback, *Eucalia inconstans*, from the Red River drainage. North Dakota Acad. Sci. 17: 63–64.

SIMPSON, G. G., A. ROE, AND R. C. LEWONTIN. 1960. Quantitative zoology. Harcourt, Brace and Company, New York. 440 p.

SMITH-VANIZ, W. F. 1968. Freshwater fishes of Alabama. The Paragon Press, Montgomery. 211 p.

STARKS, E. C. 1903. The shoulder girdle and characteristic osteology of the hemibranchiate fishes. Proc. U.S. Natl. Museum 25: 619–634.

SWINNERTON, H. H. 1905. A contribution to the morphology and development of the pectoral skeleton of teleosteans. Quart. J. Microscop. Sci. London 49: 363–382.

TAYLOR, W. R. 1967. An enzyme method of clearing and staining small vertebrates. Proc. U.S. Natl. Museum 122: 1–17.

WHITAKER, J. W. MS, 1968. Habitat selection and competition in Saskatchewan stickleback (*Gasterosteidae*) during the ice-free season. M.A. Thesis. University of Saskatchewan, Saskatoon, Sask. 58 p.

WHITLEY, G. P. 1950. New fish names. Proc. Roy. Zool. Soc. New S. Wales 1948–49: 44.

Chronic Toxicity of Copper to the Fathead Minnow (*Pimephales promelas*) in Soft Water

DONALD I. MOUNT[1] AND CHARLES E. STEPHAN

Federal Water Pollution Control Administration
United States Department of the Interior, Newtown Fish Toxicology Laboratory
Cincinnati, Ohio 45244

MOUNT, DONALD I., AND CHARLES E. STEPHAN. 1969. Chronic toxicity of copper to the fathead minnow (*Pimephales promelas*) in soft water. J. Fish. Res. Bd. Canada 26: 2449–2457.

The maximum acceptable toxicant concentration of copper for the fathead minnow (*Pimephales promelas*, Rafinesque) in water having an EDTA hardness of 30 mg/liter (as $CaCO_3$) was found to be between 0.13 and 0.22 of the 96-hr TL_m value, using survival, growth, and reproduction to evaluate effect. In an earlier study the application factor for copper in water with an EDTA hardness of 200 mg/liter (as $CaCO_3$) was found to be between 0.03 and 0.08 for the same species. Suggestions are made that should increase the accuracy and precision of future determinations of application factors.

Received November 6, 1968

INTRODUCTION

ESTIMATING CONCENTRATIONS of toxic pollutants that will be safe for desirable aquatic life, especially fishes, has been a subject of considerable investigation. Many responses have been investigated in bioassays with fish (Warner, 1967). Toxicant-induced deviations from normal resulting from exposure to nonlethal concentrations have usually been found; yet in pollution control work, and in establishing water quality standards, there has been reluctance to accept such test results as a basis for estimating safe concentrations unless these deviations can be shown to affect the harvestable crop of the species.

In addition, because the toxicity of most pollutants varies with water characteristics and fish species, no one safe concentration is likely to be applicable to all water bodies. For this reason, many investigators feel that a safe concentration should be established for each body of water and each pollutant of concern. The problem then is how to determine the safe concentration for the water and the species of concern without waiting for the results of time-consuming and expensive research.

Mount and Stephan (1967) proposed that an application factor, calculated by dividing the "maximum acceptable toxicant concentration (MATC)" by the 96-hr TL_m value, be used to determine safe concentrations of toxic pollutants. The MATC is the highest continuous concentration that does

[1]Present address: National Water Quality Laboratory, 6201 Congdon Boulevard, Duluth, Minnesota 55804, USA.

Printed in Canada (J1277)

not significantly decrease the laboratory fish production index, which takes into account survival, growth, and reproduction. Further, it was suggested that the application factor for a given toxicant, experimentally determined for one species of fish in one water, might be applicable to other waters and other species. If this were true, the maximum safe concentration of a given toxicant could be estimated by determining the 96-hr TL_m value for the species of concern in the water of concern and multiplying by the previously determined application factor for the toxicant. This application factor cannot take into account the possibility that stress caused by the toxicant may cause a shift in species composition in a water body or that some food organisms may be greatly affected by the toxicant. However, this approach would allow one to estimate an upper limit for the safe concentration of the pollutant of concern. Hayes (1967) proposed the use of a "chronicity factor" to compare single dose experiments with multiple dose ones. His proposed chronicity factor and the application factor are similar in many respects.

The main objective of this test was to determine the application factor for fathead minnows and copper in a soft water, in which the acute toxicity of copper would be greater than in the hard water previously used (Mount, 1968). Although hardness is used to distinguish the waters used in the tests, no attempt was made to control the associated characteristics such as *p*H, acidity, and alkalinity.

MATERIALS AND METHODS

The methodology for this experiment was generally similar to that described by Mount (1968). A proportional diluter described by Mount and Brungs (1967) was used to deliver five concentrations of copper and a control. The chemical metering device described by Mount and Warner (1965) was used to deliver the copper sulfate solution, which was stored in a mariotte bottle. The test chambers (12 × 12 × 24 inches) were made of double strength window glass and contained a compartment (6 × 6 × 12 inches) in one corner for fry growth studies. Water flowed from the main compartment through one stainless steel screen into the fry compartment and then through a second screen into a drain. Just before egg production began, the adults in each test chamber were divided into two groups (A and B) and were kept separated by a plastic screen placed across the middle of the test chamber. Glass and screen were glued together with silicone rubber glue. One 60-w incandescent bulb was suspended 10 inches over each test chamber.

The water was a mixture of pond water and carbon-filtered, deionized Cincinnati tap water. After the test chambers were divided by a screen, influent water was introduced into both ends. Initially, 85 ml/min was delivered to each chamber. This was increased to 160 ml/min as the fish grew and required more oxygen. Acidity, alkalinity, *p*H, and conductivity were determined on two different chambers each week. Dissolved oxygen was determined in each chamber each week. Temperature was continuously recorded in one chamber for 7-day periods. Methods described by the American Public Health Association (1960) were used throughout.

Three methods of copper analysis were employed because of the poor precision of the measurements. Polarographic analyses (Mount, 1968) were made for the entire period; colorimetric analyses using cuprizone (Middleton, 1965) were begun on January 26, 1967; and atomic absorption analyses were begun on May 1, 1967.

For the colorimetric method the copper in a 1-liter sample was collected on a cation resin column, eluted with 3 N HCL, and evaporated to dryness. The sample was moistened with 1 ml of 10% concentrated HNO_3, dissolved in 10 ml of 2% citric acid monohydrate, buffered with 10 ml of 5% concentrated NH_4OH, and allowed to react with 5 ml of 0.2% cuprizone in a 50%

methanol solution. The absorbance at 600 mμ was read in 5-cm cells after 30 min. Then 1 drop of ethylenediamine and 2 drops of 10% ascorbic acid were added to destroy the copper–cuprizone complex, and the absorbance was read at 600 mμ 10 min later. The difference in absorbance between the first and the second reading was compared with that for standards run at the same time. Spiked water samples gave quantitative recovery within experimental error. The copper–cuprizone complex formed in 10 min and was stable for over an hour. The minimum detectable concentration of copper was 1 μg/liter in the original sample.

The atomic absorption analyses were made by evaporating the sample to dryness, adding 5 ml of 1% concentrated HCl, and aspirating the mixture into the spectrophotometer. Deionized water standards were used for a standard curve. Several spiked-test water samples gave the same results as deionized water samples and the curves were considered the same for both test and deionized water. The minimum detectable concentration of copper was 2 μg/liter in the original sample.

The exposure was begun on October 26, 1966, using fry collected from the Newtown Fish Farm, Ohio Division of Wildlife. They were 10–20 mm total length and approximately 6 weeks old. Twenty individuals were randomly assigned to each test chamber. A long photoperiod of 16 hr was used to encourage rapid growth. On March 14, 1967, just after the fish had begun to spawn, the photoperiod was decreased to 12 hr. It was then increased 1 hr every 2 weeks until the photoperiod again reached 16 hr. This period was then maintained until the end of the test on September 18, 1967. The reduction in photoperiod induced the fish to revert from spawning condition to normal winter appearance and condition. The subsequent increasing of the photoperiod resulted in sexual development and an abrupt beginning of egg laying on May 29, 1967.

Some live food was present in the water, particularly cladocerans and tendipedids. Diatoms and green algae grew abundantly on the tank bottoms except in the high test concentration of 18.4 μg/liter of copper. Light intensity was adjusted so that little filamentous algae grew. Dry trout chow was fed ad libitum daily.

Three spawning tiles were placed in each half of the chambers. Eggs were removed each day before 10:00 AM. Any number of eggs was considered a complete spawning if no spawning activity was in progress. Eggs were incubated in the same apparatus as described by Mount (1968). On the 7th day after fertilization, "hatchability" was calculated as the percentage of fry surviving from the 50 eggs tested.

Two groups of approximately 50 fry from the "hatchability" measurements were placed into the fry compartments for growth measurement. There was a considerable amount of debris on which the fry could feed. Supplemental food, fine trout chow, was provided.

Prior to measurement, fish were anesthetized in 2 ml of paraldehyde per liter of water. This concentration gave complete immobilization without stopping ventilation and was no more than $\frac{1}{15}$ of the lethal concentration. This anesthetic was recommended by Mr Paul Osborn, Ozark Fisheries, Stoutland, Missouri, and appears to have great utility for such purposes. The fish were then drawn into a small glass tube and measured to the nearest millimeter.

Both static and continuous-flow 96-hr TL_m values were determined. American Public Health Association (1960) methods were used for static bioassays with five fish per 10 liters and 10 fish per concentration. Similar methods were used for continuous-flow bioassays with 10 fish per concentration. The short-term tests were run in the same water as was used for the long-term tests and the temperature was maintained at 25 ± 1 C.

In previous years, pathological examinations, limited by our capability, had no value in demonstrating effects in fish that appeared normal. Therefore, no pathological examinations were made.

PHYSICAL AND CHEMICAL CONDITIONS

The means and standard deviations for the various methods of analysis for copper (Table 1) show good agreement. A summary of the routine water chemistry analysis is given in Table 2. The mean of 243 determinations of hardness was 31.4 mg/liter (as $CaCO_3$) and the standard deviation was 2.8 mg/liter.

TABLE 1. Concentrations (μg/liter) of copper found by three methods in composite samples of test waters.

Method	Nominal copper concn (*μg/liter*)					
	20	10	5	2.5	1.3	0.0
Polarographic	18.1±4.5[a]	10.7±2.8	7.6±3.0	4.6±1.6	4.0±1.7	2.7±1.1
	42[b]	42	41	42	41	42
Colorimetric	18.1±5.1	10.4±2.8	7.9±2.4	5.4±1.1	4.4±0.8	3.8±1.4
	30	35	35	35	35	34
Atomic absorption	19.3±4.3	10.8±2.2	7.6±2.2	5.4±1.3	5.3±2.3	3.6±1.1
	19	19	19	19	19	19
Avg	18.4±4.7	10.6±2.7	7.7±2.7	5.0±1.4	4.4±1.8	3.3±1.3
	91	96	95	96	95	95

[a]Standard deviation.
[b]Number of analyses.

TABLE 2. Chemical analyses of test waters.

Factor	No. of samples	Copper concn (*μg/liter*)				
		18.4	10.6	7.7	5.0	4.4
Dissolved oxygen (*mg/liter*)	240	7.9±1.0[a]	7.6±1.4	7.2±1.5	7.3±1.5	7.3±1.5
*p*H	80	7.2±.3	6.9±.2	7.0±.3	7.0±.2	7.1±.2
Alkalinity (*mg/liter as* $CaCO_3$)	80	30±2.5	30±1.7	30±1.6	30±3.4	31±2.1
Acidity (*mg/liter as* $CaCO_3$)	80	4.3±1.8	6.4±2.3	5.6±2.1	4.8±1.3	4.8±2.1
Specific conductivity (*mhos*)	80	77±2	74±2	76±7	79±8	79±11

[a]Standard deviation.

Temperature did not vary greatly because of the modifying influence of laboratory air temperature on the water temperature. The mean weekly temperature was 19 C at the beginning of the test on October 26, 1967. The weekly mean remained at this value plus or minus 1 C until April. During this month it rose to 23 C and through May it rose to 24–25 C and remained there until the end of the test in September. The difference between the weekly minimum and maximum was 4–5 degrees C during winter and 3–4 degrees C during spring and summer.[2]

[2]Five years chronic testing had indicated that seasonal temperature fluctuations are not necessary to initiate spawning of fathead minnows. This conclusion is consistent with observations in natural ponds in Ohio where fish that hatched in May spawned the same summer. It seems certain that this species will spawn whenever the temperature is high enough and the photoperiod long enough.

RESULTS

The highest concentration tested, 18.4 μg/liter, killed half the fish, retarded the growth and sexual development of the survivors, and prevented spawning (Table 3). Survival, growth, and reproduction were similar in all

TABLE 3. Survival, growth, and reproduction data for original test fish.

	Copper concn (*μg/liter*)									
	18.4		10.6		7.7		5.0		4.4	
	A	B	A	B	A	B	A	B	A	B
Survival (%)	50	50	90	80	90	100	90	100	90	70
No. males										
Mature	1	0	2	3	4	3	4	5	2	1
Immature	1	1	0	0	0	0	0	1	0	0
No. females										
Mature	0	1	7	5	5	7	5	4	7	6
Immature	3	3	0	0	0	0	0	0	0	0
Length (*mm*) at termination										
Male	48	48	66	60	69	65	64	65	70	58
Female	37	37	54	53	56	54	52	52	54	53
Spawnings/female	0	0	3.9	4.6	2.8	3.6	4.8	2.8	2.1	6.5
Hatchability (%)	–	–	70(13)*	32(10)	47(8)	68(9)	65(10)	72(7)	68(5)	66(20)
Total eggs produced	–	–	5113	4005	898	1345	5527	1506	1183	5988
Eggs/spawning	–	–	189	174	64	174	230	140	79	153

*Number of spawnings checked.

lower concentrations of copper. In these concentrations copper caused no detectable effect on spawning behavior, on the ability of the males to care for the eggs, or on the initiation and distribution of egg production.

Fry survived and grew equally well in the various concentrations where they were produced (Table 4) and fry from unexposed parents survived and grew equally well when placed in the various concentrations of copper except the highest, where survival was zero (Table 5).

The mean 96-hr TL_m of two continuous-flow bioassays, with two replicates each was 75 mg/liter based on measured concentrations. The value in two static tests was 84 mg/liter based on nominal concentrations. The application factor, based on the static test (the static test was used for the hard-water application factor) lies between 18.4/84 and 10.6/84 or between 0.22 and 0.13.

DISCUSSION

The low test concentration (4.4 μg/liter) was used as the control because most of the fish in the tank to which no copper was added were killed accidentally. The analyses (Table 1) did not indicate a very large difference in copper

TABLE 4. Growth and survival of two groups of fry produced and grown in various copper concentrations for 30 days.

		Copper concn (*µg/liter*)				
	Group	18.4	10.6	7.7	5.0	4.4
Survival (%)	1	–	44	54	94	46
	2	–	80	62	80	76
Final length (*mm*)						
Mean	1	–	12.0	11.3	14.8	13.9
	2	–	16.5	11.4	12.3	10.3
Range	1	–	8–19	9–14	10–20	9–19
	2	–	13–19	9–15	9–17	8–13

TABLE 5. Growth and survival of fry in various copper concentrations from unexposed parents.

	Copper concn (*µg/liter*)				
	18.4	10.6	7.7	5.0	4.4
Survival (%) of 45 fry	0	62	73	64	78
Length (*mm*) after 60 days					
Mean	–	16.4	14.5	15.3	15.0
Range	–	11–22	9–25	10–22	9–25
Survival (%) of 10 fry[a]					
90 days	–	100	100	100	100
120 days	–	90	100	100	80
Length (*mm*) of 10 fry					
60 days (initial)	–	15	15	15	16
90 "	–	25	25	26	24
120 "	–	33	35	37	36

[a]After 60 days, 10 fry closest to the mean size were tested for an additional 60 days.

concentrations between the intended control and the low test concentration and the biological data (Table 3) were similar for the 4.4-, 5.0-, 7.7-, and 10.6-µg/liter concentrations, suggesting no adverse effect at the 4.4-µg/liter concentration.

In the test with fathead minnows and copper in hard water (Mount, 1968), 95 µg/liter killed almost half the fish, retarded sexual development, and prevented spawning. The second highest concentration, 33 µg/liter, did not affect survival or the physical appearance of the fish but did prevent spawning. The fish in the lower concentrations, 14.5 and 5.8 µg/liter, were indistinguishable from those in the control tank (4.4 µg/liter) in the hard-water study in terms of survival, growth, and reproduction. In the test with fathead minnows and copper in soft water, 18.4 µg/liter reduced survival by 50% and prevented spawning. The fish in the lower concentrations survived,

grew, and reproduced equally well. One can assume that some concentration between 18.4 and 10.6 μg/liter in the soft water would have produced results equivalent to those found in 33 μg/liter in hard water. However, since there is no information available on the range of partial effects of copper on spawning, it is not possible to estimate whether it would be closer to 18.4 or 10.6 μg/liter.

Because it is not possible to interpolate between concentrations, it is not possible to determine the MATC, and thus the application factor, precisely. Rather, one can say that they lie within certain limits. For the soft-water test the application factor was between 0.13 and 0.22 whereas for the hard-water test it was between 0.03 and 0.08 (erroneously given as 0.07 in Mount and Stephan, 1967). These results (Table 6) indicate that hardness may affect

TABLE 6. Summary of application factor data.

	Soft water	Hard water (Mount, 1968)
Limits on MATC[a] (*μg/liter*)	18.4–10.6	33.0–14.5
96-hr TL_m (*μg/liter*)		
Static, based on nominal concentration	84	430
Continuous-flow, based on measured concentration	75	470
Limits on application factor		
Using static 96-hr TL_m	.13–.22	.03–.08
Using continuous-flow 96-hr TL_m	.14–.24	.03–.07

[a]MATC = maximum acceptable toxicant concentration.

the application factor for copper. However, the fact that the application factors compared here may differ by as little as a factor of 1.6 suggests that this difference may have been due to one or more of the following reasons: (1) different lots of fish were used in the long-term and in the short-term tests in both the hard-water and in the soft-water experiments and the different lots may have varied in their sensitivity to copper; (2) quantitative comparisons were made using results of a long-term and a short-term test, neither of which has accuracy and precision limits defined; (3) all of the copper in the water may not have been in toxic form, and the percentage of nontoxic copper in the water may have varied from test to test.

In light of these three considerations, the two determinations of the application factor for copper and fathead minnows compared here are not conclusive evidence that hardness does or does not affect the application factor for copper and fathead minnows. We feel that judgment must be withheld until additional refined tests are completed. These refined tests must include

the following considerations, some of which were not explicitly taken into account in previous work but must be part of any test designed to check the validity of the application factor. (1) The long-term and the short-term bioassays for the determination of any one application factor must be conducted with fish from the same lot. (2) Very young fish and sexually maturing, mature, spawning, or spent fish should not be used for the 96-hr bioassays. (3) The 96-hr bioassay should be conducted on a continuous-flow basis and the calculated 96-hr TL_m should be based on measured toxicant concentrations. (4) The dilution factor (the number by which one concentration is multiplied to obtain the next lower concentration) for the long-term bioassays should not be lower than 0.5. (5) Means must be devised to determine and measure what portion of the toxicant is actually toxic.

We believe that this study has furnished evidence that the application factors for copper are similar enough in two different waters to warrant further investigation of the entire hypothesis. We hope that others will assist us in evaluating the validity of this method. Studies are now in progress with other species and other toxicants and work has begun to field test this approach in a natural stream.

The application factor approach offers the possibility of estimating maximum safe concentrations of toxicants with an acceptable amount of effort. The methodology is available to use the end points of survival, growth, and reproduction for the determination of maximum acceptable toxicant concentrations and application factors. If it can be shown that this application factor is not greatly affected by the variables that often affect toxicity, fish toxicologists will have a valuable tool for the determination of maximum safe concentrations of toxicants. Even if these experimentally determined application factors lack optimum precision, their use is still preferable to the use of arbitrary application factors such as one-tenth, a value that is apparently 50 times too large for cadmium (Q. H. Pickering, unpublished data; J. G. Eaton, unpublished data).

ACKNOWLEDGMENTS

The authors wish to thank especially Mr James Dryer for routine determination of some copper analyses and calculation of means and standard deviations; Mr Kermit Figart for routine chemistry determinations and daily assistance; Mrs Eleanor Martin for some copper analyses; and Messrs Quentin Pickering and John Eaton and Dr William Brungs for assistance with egg counting and servicing of system on weekends and holidays and criticism of the manuscript.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION. 1960. Standard methods for the examination of water and wastewater. 11th ed. Am. Public Health Assoc., New York.

HAYES, W. J., JR. 1967. The 90-dose LD_{50} and a chronicity factor as measures of toxicity. Toxicol. Appl. Pharmacol. 11: 327–335.

MIDDLETON, K. R. 1965. Colorimetric determination of copper in plants. Analyst 90: 234–240.

MOUNT, D. I. 1968. Chronic toxicity of copper to fathead minnows (*Pimephales promelas*, Rafinesque). Water Res. 2: 215–223.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–29.

MOUNT, D. I., AND C. E. STEPHAN. 1967. A method for establishing acceptable toxicant limits for fish malathion and the butoxyethanol ester of 2,4-D. Trans Am. Fish. Soc. 96: 185–193.

MOUNT, D. I., AND R. E. WARNER. 1965. A serial dilution apparatus for continuous delivery of various concentrations of materials in waters. U.S. Dept. Health, Education Welfare, Public Health Serv. Publ. 999-WP-23. 16 p.

WARNER, R. E. 1967. Bioassays for microchemical environmental contaminants. Bull. World Health Organ. 36: 181–207.

Seasonal Distribution, Constitution, and Abundance of Zooplankton in Lake Erie[1]

CHARLES C. DAVIS

Marine Sciences Research Laboratory and Department of Biology
Memorial University of Newfoundland, St. John's, Nfld.

DAVIS, CHARLES C. 1969. Seasonal distribution, constitution, and abundance of zooplankton in Lake Erie. J. Fish. Res. Bd. Canada 26: 2459–2476.

Zooplankton samples, collected by vertical hauls from near bottom to the surface, were obtained from 28 stations along the length of Lake Erie in October 1967. A partial similar series, confined largely to the Western Basin because of ice conditions, was collected in January 1968. The results of the analyses are tabulated as numbers of individuals per m^3, and are discussed. Comparisons are made with results previously published for a comparable series of stations visited in July 1967.

As in July, distinct differences were to be seen among the three major basins (Western, Central, and Eastern) in the October results. These differences are summarized. In July an expected decrease in zooplankton, such as would be predicted from lake morphology, amount of runoff from rivers, etc., occurred from west to east in the lake. In October, however, the zooplankton of the Western Basin was extremely impoverished, and the expected distribution of biomass was reversed. It is shown, on the other hand, from work done by others on samples taken from the same series of stations in October, that phytoplankton, chlorophyll *a*, and seston exhibited the expected distribution, emphasizing the danger of judging an ecosystem by the examination of only limited components of the community and at single periods of the year.

In July the bulk of the zooplankters consisted of large cladocerans (especially daphnids) and copepods. In October there were much larger populations of rotifers and of small cladocerans (*Bosmina*, *Chydorus*). Copepods were relatively sparse. The January samples were characterized by greater numbers and a greater variety of diaptomids than in October, and the rotifers remained very important. All of the January samples contained many partially decayed microcrustaceans. This was interpreted as indicating a seasonal die-off associated with the very severe weather of the period.

Received May 31, 1969

INTRODUCTION

THE ZOOPLANKTON has seldom been thoroughly studied quantitatively in any of the St. Lawrence Great Lakes, as shown in a recent review (Davis, 1966). Although in this respect more attention has been paid in the past to Lake Erie than to any of the other lakes, nevertheless there is only one publication dealing quantitatively with samples collected in all three of the Lake Erie basins, taken during the same period of time, and using uniform collection and enumeration techniques. The study in question (Davis, 1968) reported the results obtained from the analysis of a series of vertical tows collected

[1]Studies in biology from Memorial University of Newfoundland No. 169.

Printed in Canada (J1482)

in July 1967 by the U.S. Federal Water Pollution Control Administration (U.S.F.W.P.C.A.), Lake Erie Program Office, Cleveland, Ohio. The only other previous quantitative studies of a wide range of Lake Erie zooplankters that have been published are Chandler (1940), Wright et al. (1955, MS, no date), and Davis (1954, 1962).

Zooplankton samples were collected at the same series of stations as in July 1967 by the U.S.F.W.P.C.A., Cleveland Office, in October 1967, and a partial series was taken in January 1968. The samples were kindly made available to the author for analysis.

METHODS

As in the study of the July 1967 samples, vertical zooplankton samples were obtained from 2 m above bottom to the surface by use of an open conical plankton net 0.5 m in diameter at the mouth, constructed of no. 20 bolting silk. Numbers of animals per m^3 were calculated, assuming that the net filtered the entire column of water in its vertical journey. All samples were preserved in formaldehyde and stored in 2-liter plastic containers until analysis. The weaknesses of these methods were discussed in Davis (1968).

Zooplankton samples were collected from a total of 30 stations, most located towards the center of the lake, approximately along the international boundary between the United States and Canada (Fig. 1). Of these stations, samples were obtained from 28 between October 17 and

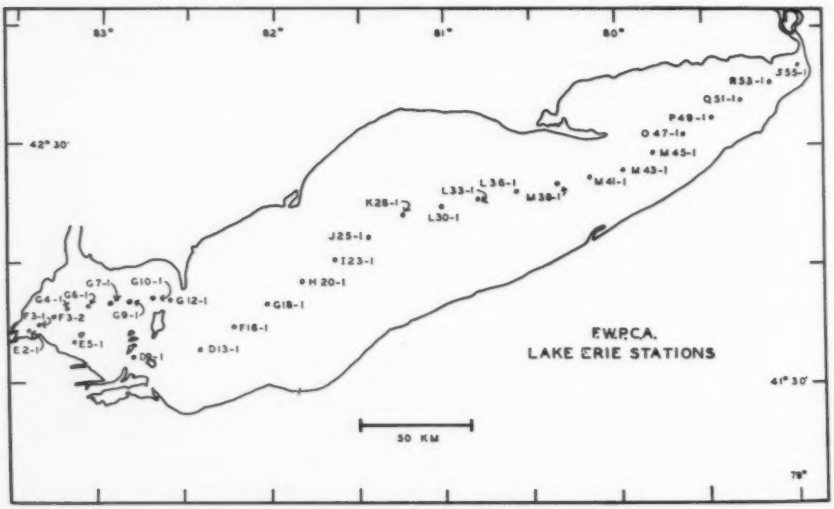

FIG. 1. Lake Erie, showing the three basins and the location of stations occupied in October 1967 and January 1968. Station numbers are those assigned by the U.S.F.W.P.C.A., Lake Erie Program Office, Cleveland, Ohio.

23, 1967. A similar trip planned for January, using a U.S. Coast Guard icebreaker ran into such severe icing conditions that the ship became locked in the ice and could not continue the voyage. Samples were obtained, however, between January 9 and 13, 1968, at nine Western Basin stations and at three of the western-most stations in the Central Basin.

After adjusting each sample to a known volume, appropriate aliquots, taken after thorough mixing, were analysed by use of a compound microscope (magnifying 35, 100, and 430×) and a stereozoom stereoscopic microscope (magnifying from 7 to 60×). Aliquot volumes used were 10 ml or multiples thereof examined in Syracuse watch glasses, 1 ml or multiples thereof examined in Sedgewick-Rafter cells, and 0.16 ml examined under standard cover slips on ordinary microscope slides. Aliquot sizes were chosen, depending upon the abundance of the several zooplankters present in each sample.

The animals, where possible and feasible, were analysed to species, and otherwise to genera. A significant proportion of the rotifers and some protozoans, however, were distorted beyond recognition by the preservative. In addition, to analyse the copepod nauplii and younger juvenile cyclopoids even to genus would have been excessively time-consuming, as would the determination of juvenile diaptomids, so that these organisms are simply reported as "copepod nauplii," "juvenile cyclopoids," and "juvenile *Diaptomus*." Inasmuch as only a single species of *Eurytemora* occurs in Lake Erie, however, copepodid stages of this form could be recognized easily, and are reported.

In all of the samples collected during the January cruise, a large number of the cladocerans and copepods were in poor condition, and often partially decayed. It was obvious that they had been moribund at the time of preservation. Most of the more delicate forms, however, such as protozoans and rotifers — and indeed, many cladocerans and copepods as well — were in sound condition, so it is believed that the mortality was due to the severe weather conditions of the period, rather than to faulty preservation. Only sound specimens were counted.

RESULTS

Protozoa

The distribution of the protozoan zooplankters is summarized in Table 1. The total numbers were comparatively small both in October and in January. As in July 1967, the greatest diversity of species occurred in the Western Basin in October. On the other hand, the diversity was reduced to two forms in January 1968 in the Western Basin; approximately the same quantities of the same forms occurred in the only Central Basin stations that could be occupied.

Compared with the huge numbers of *Vorticella* spp. encountered in the July cruise (up to $146 \times 10^6/m^3$), the maximum in October was only $15 \times 10^3/m^3$. In January there was a higher average, and the maximum was $95 \times 10^3/m^3$. The major species of this genus in July also appeared to differ from those occurring in the 2 months under consideration here, for the vast majority of the July specimens had a rather short stalk, whereas the stalk was very long in most October and January representatives. Furthermore, the distribution in October varied radically from that in October, for in the latter month the genus was almost entirely confined to the Western Basin, whereas in July it occurred at most stations in all three basins, attaining its maximum in the eastern portions of both the Central and Eastern basins. In January 1968, *Vorticella* was common at all but one of the stations that were visited. There was no clear correlation in October and January between phytoplankton abundance and numbers of *Vorticella*, in contrast to the situation in July.

In July, *Epistylis* sp. had been confined to two stations in the Western Basin. In October it was found, though in distinctly smaller numbers, at most stations in the Western Basin, and at other scattered stations, especially in

TABLE 1. Numbers of protozoans per m^3 encountered during October 1967 and January 1968. Blank = not encountered; + = seen, but in minimal numbers.

Station	Species					
	Vorticella spp.	*Epistylis* sp.		*Codonella cratera*	Unidentified ciliates	*Difflugia* sp.
		Colonies	Cells			
Oct. 1967 — West Basin						
F 3–2	+	1,800	28,100	1,800		4,500
G 4–1	6,800	+	+	+		
G 6–1	15,000			3,760	6,270	
G 7–1	2,200	2,200	26,000	3,260		+
G 9–1	4,100	+	+	3,100		
G 10–1		1,800	21,700	5,430	2,700	+
G 12–1	7,240			1,800		
E 5–1	+	8,660	64,900	6,490		
D 9–1				1,480		
Central Basin						
D 13–1		+	+	2,220		
F 16–1				4,890		
G 18–1				830		
H 20–1						
I 23–1				+		
J 25–1						
K 28–1						
L 30–1						
L 33–1				+		
L 36–1						
M 38–1				5,100		
East Basin						
M 41–1				5,680		
M 43–1		+	+	4,070		
N 45–1		+	+	8,390		
O 47–1				6,900		
P 49–1				8,780		
Q 51–1				860		
R 53–1	+	+	+	1,530		
S 55–1		+	+	5,700		
Jan. 1968 — West Basin						
E 2–1	75,100					
F 3–1	89,300			+		
F 3–2	91,700			+		
G 4–1	57,000					
G 7–1	94,500					
G 10–1	13,600			+		
G 12–1	27,200			+		
E 5–1	50,500					
D 9–1	16,800					
Central Basin						
D 13–1	61,100			+		
F 16–1	86,600			2,040		
G 18–1						

the Eastern Basin. This form was not observed in January, nor was *Difflugia*, which occurred entirely in the Western Basin in October and mainly so in July. As previously mentioned (Davis, 1968) both *Epistylis* and *Difflugia* are usually considered to be benthic forms, but both occur regularly in Lake Erie plankton, and although *Difflugia* may well be tychopelagic, *Epistylis* may well be holoplanktonic inasmuch as this has been shown by Rylov (1935) and Nipkov (1956) to be the case for the European species *E. rotans*.

Codonella cratera was rather impoverished in July, at which time it was found only at a few stations in the Western Basin. In October it was distinctly more abundant, and was encountered at all Western and Eastern basin localities. It was lacking or impoverished only in the central portion of the Central Basin. The maximum was $8.8 \times 10^3/m^3$ at station N45-1 in the Eastern Basin. This is small, however, compared with the results of others, especially the maximum of $1126 \times 10^3/m^3$ reported in the Central Basin near Cleveland by Davis (1954). The numbers encountered in January 1968 were mostly insignificant (Table 1), although the species was seen at half of the stations.

Rotifera

The distribution of rotifers is shown in Table 2. Rotifers were relatively very abundant in the October samples, when they were far more numerous than any other zooplankters. Biomasses were not measured in this study, but it is doubtful that rotifers exceeded crustaceans in biomass.

In the Western Basin rotifers averaged nearly $129 \times 10^3/m^3$ at the nine stations in October. This is slightly less than the July average of $135 \times 10^3/m^3$. On the other hand, in the Central and Eastern basins they averaged $118 \times 10^3/m^3$ and $153 \times 10^3/m^3$ respectively. Hence they were distinctly more abundant in these basins than in July when the average for the Central Basin was $54 \times 10^3/m^3$, and that for the Eastern Basin was only $42 \times 10^3/m^3$.

The variety of rotifer species in the Western Basin, in October as in July, was decidedly greater than elsewhere.

By January, as might be expected, the number of rotifers had been decimated, so that the average for all Western Basin stations was only $24.5 \times 10^3/m^3$, or only one-sixth of the October average. The limited results from the western portion of the Central Basin suggest that in this deeper water area the rotifers still maintained relatively high populations, although reduced compared with October. As in the study of the July 1967 samples, soft-bodied rotifers could not safely and consistently be identified even to genus because of the killing agent that was used, and hence they are listed as "unidentified rotifers." Unfortunately such forms were rather abundant, both in October and in January, being the dominant forms in some of the January tows (e.g., at stations F3-2 and G12-1 in the Western Basin).

Of the identifiable rotifers, *Brachionus angularis* was rarely encountered. It occurred, however, at three stations, two of which were in the Western Basin, and one in the western-most portion of the Central Basin. In the July

TABLE 2. Numbers of rotifers per m³ encountered during October 1967 (A: [illegible]

Species		Station												
		E2–1	F3–1	F3–2	G4–1	G6–1	G7–1	G9–1	G10–1	G12–1	E5–1	D9–1	D13–1	F16–1
Asplanchna sp.	A				8,150	1,130		310	720	540	870	3,330	220	1[illegible]
" "	B													1[illegible]
Brachionus angularis	A							+					+	
" "	B		+											
Brachionus sp.	B	+							+					[illegible]
Brachionus calyciflorus	A			630	24,450	5,020	2,170		+			2,220		[illegible]
" " ovig.	A			270	8,150	+	2,170							
" " 2 eggs	A			+	4,070	+								
" " 3 eggs	A			+	+	+								
Conochiloides sp.	A				+				1,810	+				4,[illegible]
Filinia longiseta	A													
" "	B											190		
Kellicottia longicornis	A													[illegible]
" "	B			+					1,810	1,700	490	+	1,340	1,[illegible]
" " ovig.	A													1,[illegible]
" " ovig.	B								+	+				270
Keratella cochlearis	A			5,430	27,200	15,000	19,600	49,900	11,800	29,900	62,800	10,400	11,900	35,[illegible]
" "	B		4,070	7,000	2,040		2,170		5,430	5,090	330	1,060	20,140	29,[illegible]
" " ovig.	A			4,530	10,900	3,760	18,500	29,500	2,720	9,960	15,200	2,220	2,960	6,[illegible]
" " ovig.	B		+		1,360				910	+	+	290	4,860	3,[illegible]
" " 2 eggs	A							+						
Keratella quadrata	A				2,720			+	+		4,330			
" "	B	7,000	2,910	3,180	8,150		7,060		4,070	6,790	+	480	34,700	38,[illegible]
" " ovig.	A				+									
" " ovig.	B	3,180	3,490	1,910	4,750		2,170		2,260		+		19,400	7,[illegible]
" " 2 eggs	A				2,720									
Polyarthra vulgaris	A			10,900	23,100	13,800	16,300	21,400	37,100	54,300	67,100	35,600	55,600	77,[illegible]
" "	B		1,160				+		2,720	+	330		6,250	7,[illegible]
" " ovig.	A												+	[illegible]
Polyarthra spp.	A			3,620	5,430	3,760	2,170	4,070	4,530	5,430	13,000	5,930	3,700	6,[illegible]
" "	B		1,750							+			3,470	7,[illegible]
Synchaeta stylata	A			+		2,510	17,400	23,400	2,720	15,400	112,600	5,190	36,300	
Synchaeta sp.	A				+	+								
" "	B												1,340	
Trichocerca sp.	A			+	+		+		+	2,720	+			[illegible]
Unidentified rotifer	A			3,620	24,400	6,270	10,900	12,200	14,500	77,000	52,000	43,700	62,200	2,[illegible]
" "	B	1,910	8,730	63,700	4,070				5,430	30,600	1,790	290	20,800	15,[illegible]
" " ovig.	A												+	
Rotifer eggs (free)	A			1,810	5,430					7,240	13,000		14,800	20,[illegible]
" "	B	+	2,330	+						4,240			1,340	6,[illegible]

samples this species was reported to be rather abundant, but nearly confined to the Western Basin; furthermore, all previous records of the species from Lake Erie have been from the Western Basin only.

Brachionus calyciflorus, which was seen neither in the July nor the January samples, was common in the Western Basin in October, with a maximum of $24.5 \times 10^3/m^3$ at station G4-1. At the western-most stations the species was reproducing vigorously, some females carrying as many as three eggs.

Conochilus unicornis, which was very abundant in July in the shallower areas of the Western and Eastern basins, was not seen either in October or January, confirming it as a summer species (Ahlstrom, 1933; Chandler, 1940; Davis, 1954, 1962). On the other hand, during October *Conochiloides* sp. was

967 (A) ...uary 1968 (B). Blank = not encountered; + = seen, but in minimal numbers.

| | | |

The ubiquitous *Keratella cochlearis* remained, as in July, one of the most abundant rotifers at most stations sampled. Many of the females were ovigerous. In October there was a sharp increase in abundance compared with July. The marked concentration of the species exhibited in the Western Basin in July was no longer in evidence. The October averages for the Western, Central, and Eastern basins were respectively $36.5 \times 10^3/m^3$, $34.7 \times 10^3/m,^3$ and $43.3 \times 10^3/m^3$. This contrasts with the corresponding averages for July of $13.2 \times 10^3/m^3$, $7.2 \times 10^3/m^3$ and $1.1 \times 10^3/m^3$. In January, the Western Basin average had dropped to $3.3 \times 10^3/m^3$; the limited results from the Central Basin show a much less drastic decline.

Keratella quadrata was approximately twice as abundant in January as *K. cochlearis*. A large proportion of the specimens was ovigerous. At the two western-most stations of the Central Basin *K. quadrata* was particularly abundant, with a maximum of $34.7 \times 10^3/m^3$. This far exceeded the maximum ($14.6 \times 10^3/m^3$) found at any station in July, although the average for all the Western Basin stations ($6.4 \times 10^3/m^3$) was not very much larger than the average for the same area in July ($5.9 \times 10^3/m^3$). In October, on the other hand, *K. quadrata* was comparatively uncommon, for only at two stations in the Western Basin did it occur in greater than minimal numbers (maximum $4.3 \times 10^3/m^3$).

In October the dominant rotifer species, *Polyarthra vulgaris*, exhibited the same pattern of distribution in the lake as in July, being less common in the Western Basin, and increasing towards the east (the identification of *P. vulgaris* in Lake Erie is discussed in Davis (1968)). In the Western Basin the average was $31.1 \times 10^3/m^3$, in the Central Basin $60.5 \times 10^3/m^3$, and in the Eastern Basin $73.4 \times 10^3/m^3$, with a maximum at the two eastern-most stations (in the vicinity of Buffalo) of $122.2 \times 10^3/m^3$. The October populations were approximately twice as large in the Central and Eastern basins as those of July. In the Western Basin the increase was 15-fold. The Buffalo maximum exceeded any previously reported from Lake Erie itself, although Ahlstrom (1933) listed a maximum of $2218 \times 10^3/m^3$ (mostly *P. trigia* = *P. vulgaris*) for Terwilliger's Pond, an enclosed shallow body of water open by a narrow channel to Lake Erie. The quantities of *P. vulgaris* in January 1968 were far less than those of either July or October 1967.

Other, unidentified species of *Polyarthra* were far less common than *P. vulgaris*. Their proportional quantitative distribution in the three basins, however, was similar in October, and as far as the collections warrant a decision, also in January.

In October, *Synchaeta stylata* was common, occurring at nearly all stations in the Western Basin, and at the western-most stations in the Central Basin. Elsewhere it was found neither regularly nor in great abundance. In July the distribution had been similar, but at that time the species was entirely confined to the Western Basin and the population size was considerably smaller. The species was not encountered at all at any of the January 1968 samples.

CLADOCERA

The distribution of cladocerans is shown in Table 3. The total biomass of the members of this order was considerably reduced in October as compared with July, mainly because of a great reduction in the numbers of the larger species. On the other hand, certain smaller species had increased in abundance. In January 1968 cladocerans were of little quantitative importance.

Some of the cladoceran species were present only in small numbers, but nevertheless are of interest. Among these, as discussed in Davis (1968), the large predatory species *Leptodora kindtii* could not be sampled quantitatively by the methods used. In October no females were observed in the samples, the only adults found being males. This agrees with Andrews (1949), who showed maxima of males in the Western Basin during September and October. The lack of any specimens of this genus in the January collections also is supported by Andrews' observations.

Holopedium gibberum also was evidently lacking in January, but it occurred at 13 of the stations in October, in small numbers compared with the July samples. As mentioned in Davis (1968), all published records for this species in Lake Erie have referred only to the Central and Eastern basins, although the plankton has been studied far more frequently in the Western Basin than elsewhere. The same distribution was repeated in October.

Diaphanosoma leuchtenbergiana occurred mainly in the Central and Eastern basins in October, but more frequently and in larger numbers in the Central Basin. This contrasts sharply with the situation in July, when nearly all records were from the Western Basin.

Ceriodaphnia lacustris occurred in small numbers at six of the Central Basin stations in October. It was not seen either in July or in January. This species was determined by use of keys in Brooks (1959) and Pennak (1953). It has not previously been reported from Lake Erie, although *C. reticulata* has been reported in small numbers by Chandler (1940), Davis (1954), and Wilson (1960).

In July the bulk of the cladoceran biomass was formed by *Daphnia galeata mendotae* and *D. retrocurva*. *Daphnia galeata* was the more common of the two, and was approximately evenly distributed in the three basins, whereas *D. retrocurva* occurred in large numbers only in the Western Basin. In the present study, however, the numbers of both of these species were relatively very small in October; there were still fewer at the stations visited in January. In October, both species were nearly absent from shallow waters of the Western Basin. *Daphnia galeata* occurred in greatest numbers in the eastern portion of the Central Basin and the western portion of the Eastern Basin. *Daphnia retrocurva* was apparently absent from most stations in the Western and Central basins. There was some evidence of an increase of numbers at the (artificial) boundary between the Western and Central basins, but because of the small numbers of this species seen, this remains uncertain.

The important cladocerans, both in October and January, were the small to very small species: *Bosmina coregoni*, *B. longirostris*, and *Chydorus*

TABLE 3. Numbers of Cladocera per m³ encountered during October 1967 (A

Species		Station												
		E2-1	F3-1	F3-2	G4-1	G6-1	G7-1	G9-1	G10-1	G12-1	E5-1	D9-1	D13-1	F16
Bosmina coregoni	A			+	+		+	+	1,630	900	650	2,590	2,000	60
" "	B	+	+	+	+		+		180	+		100	+	10
" " juv.	A			360	1,220	250	+	+	910	910	650	5,930		1,4
" " "	B	+			+		+			+				
" " gravid	A				+	+	+	+	+	+	+	220	+	
" " "	B			+			+		+			+	130	
B. longirostris	A			2,720	4,210	1,130	980	2,750	12,700	5,340	23,800	6,300	8,740	
" "	B	+	+						+	170	+	+	+	3
" " juv.	A			4,710	5,030	880	2,060	3,260	13,600	9,050	54,100	14,100	22,200	
" " "	B		+	+	+						+		490	2
" " gravid	A			630	820	380	220	1,530	1,360	1,360	5,410	2,150	2,740	
" " "	B	+	+							+			130	1
Ceriodaphnia lacustris	A												+	1
" " juv.	A													2
" " gravid	A													
Chydorus sphaericus	A			180	+	+		+	1,090	540	1,730	1,040	2,960	1
" "	B		+										210	
" " juv.	A			180	540				360	180	1,520	590	120	2
" " "	B	+			+								210	
" " gravid	A				+						+	440	+	2
Daphnia galeata	A				+					+				
" "	B			+	+		+			+		+		1
" " juv.	A												+	1
" " "	B													
" " gravid	A													
" " "	B									+				
D. retrocurva	A								+		+	150	370	
" "	B													
" " juv.	A				+				270	180	+	1,260	520	1
" " gravid	A				+	+			+	+	+	370	220	
Diaphanosoma leuchtenbergiana	A									+			+	1
" " juv.	A													1
" " gravid	A													
Holopedium gibberum	A													+
" " juv.	A													
" " gravid	A													
Leptodora kindtii ♂	A								+			+		
" " juv.	A						+		+					+

sphaericus. Davis (1968) discussed the ambiguous nature of the species of *Bosmina* that was common in July. This form had the frontal hair and the pecten as described for *B. coregoni*, but no mucrones were present. The species was called *B. coregoni* based on the key in Brooks (1959), with the reservation that the mucrones were not as described. Both in October and in January this same form remained common, but a second form with sensory hair, pecten, and mucrone as described for *B. longirostris* was even more abundant. As mentioned by Davis (1968), all previous published records of the genus *Bosmina* for Lake Erie have referred only to *B. longirostris*.

The form here called *B. coregoni* had been abundant only in the Western Basin in July. In October, however, it was substantially less abundant there than elsewhere (increasing in numbers towards the eastern end of the basin).

67 (A ...ary 1968 (B). Blank = not encountered; + = seen, but in minimal numbers.

		Station																
-1	F16-1	H20-1	I23-1	J25-1	K28-1	L30-1	L33-1	L36-1	M38-1	M41-1	M43-1	N45-1	O47-1	P49-1	Q51-1	R53-1	S55-1	
00	[illegible]	500	740	1,090	970	820	840	2,400	2,550	1,160	430	670	500	3,040	1,500	2,290	1,960	
+	[illegible]																	
	1,[illegible]50	1,980	2,760	3,530	3,090	1,290	2,980	3,530	4,840	4,000	530	1,790	740	3,420	4,890	8,660	1,470	
+	+ 130	80	190	120	100	170	230	450	150	80	+	90	50	310	90	150	160	
30	+ 90																	
740	[illegible]	+		120				180	+	120	800	+	200	1,910	2,450	3,360	2,610	
+	30 970																	
200			1,550	820			140	+	610	330	330	670	280	1,630	7,290	14,300	3,750	
190	28 150																	
740		+	120	+			+		+		80	+	+	160	30	870	410	
130	18 150																	
+	[illegible]	80	+	+	100													
	[illegible]	+	+						+		+	+						
	-																	
960	15 270	3,220	3,030	3,180	920	520	5,260	4,930	2,950	620	650	1,360	930	250	260	310	160	
210	+ 170																	
120	2,[illegible]90	1,070	2,640	2,060	1,210	860	2,560	1,310	1,170	140	220	600	90	60				
210	+																	
+	2[illegible]90	330	620	80	100	260	650		100	30			+					
	- +			+	100	260	140	180	1,120	620	120	170	270	560		+	+	
	1[illegible]10																	
+	1[illegible]+	290	620	820	150	170	370	400	1,530		270	770	280	750	210	260	570	
	-	+		80	+	+	90	90	260	+	80	240	190	90	+	100	+	
	+																	
370	- +		+						150		+	+	+	90			+	
	-																	
520	1[illegible]+		230	+		170	190	90	560	540	80	190	240	470	90	310		
220				+					+				+	+	+	+	240	
+	[illegible]		120	+	+	+	+	90	100		40		+		+		+	
	[illegible]		+					+	150			+		+		+		
						+	+							+				
	+		+		+		+		+	50		+	+	+	+			
						+					+							
								+	+			+	+					
			+					+				+						
	+	+		+					+					+		+		

Bosmina longirostris was, on the other hand, far more common in the Western Basin than elsewhere, being present in negligible numbers or apparently absent entirely from most Central Basin stations. It should be noted that often *B. longirostris* is considered to be characteristic of eutrophic, and *B. coregoni* of oligotrophic waters (e.g., see Beeton (1966)). The present results and those for the July samples throw only confusion on this designation.

The very small cladoceran, *Chydorus sphaericus*, occurred only in small numbers in July, but in October it was common, especially in the Central Basin, where it was reproducing actively. *Chydorous sphaericus* has previously been reported from Lake Erie several times. Although Marsh (cited in Wright et al., MS, no date) decided "the conditions in Lake Erie are not favorable for its development" (in 1929-30), and Wilson (1960) found the species only

rarely in the same years, Vorce (1882) many years previously had described it as common. Davis (1962) encountered an October maximum in 1956 amounting to 24 $\times$ $10^3/m^3$ (a total of 50.1 $\times$ $10^3/m^3$ for adults and juveniles together). The October 1967 maximum, in contrast, was only 5.9 $\times$ $10^3/m^3$ of adults (8.5 $\times$ 10^3 total/m^3) at station L33-1. Because of the small size of juveniles of this species, it is possible that a large proportion of them passed through the meshes of the net, and that they are underrepresented in the counts.

COPEPODA

The distribution of copepods is summarized in Table 4. As in the study of the July samples, time did not allow a distinction between calanoid and cyclopoid nauplii, nor between species of juvenile cyclopoids and diaptomids. Both in October and especially in January, the copepod populations were considerably reduced compared with those of July. Nauplii and juveniles also were reduced. There were a great many moribund specimens, often partially decayed, in the January samples, as mentioned above. These were not counted.

During October the most abundant copepods were the juvenile cyclopoids, fewer occurring in the Western Basin than elsewhere. *Cyclops bicuspidatus* was found only in minimal numbers in the Western Basin. It also was not very abundant in the other basins, unlike the situation in July, although in July as in October it was least abundant in the Western Basin.

In July, *C. vernalis* was confined to the Western Basin. Likewise in October most of the records were from the Western Basin, but the species occurred in the other basins as well. It must be stated, however, that very few specimens were encountered either in the October or the January samples.

Mesocyclops edax was lacking in the Western Basin in October, but was found regularly at most Central and Eastern basin stations. It was never abundant, however. The distribution was similar in July, although at that time the species was much more common. It was not encountered at all in the January samples, confirming Andrews' (1953) judgement that it is a warmwater species, absent from Lake Erie when the water temperature is lower than 8 C.

Tropocyclops prasinus was not seen in July, but it occurred regularly in the Western and Eastern basins in October, and in the Western Basin in January. Previous records of this species from Lake Erie have usually indicated it as present in small numbers, with an autumnal maximum. Chandler (1940) stated that it occurred in the Western Basin with a maximum in August of 4 $\times$ $10^3/m^3$. Davis (1962) observed a maximum in the Central Basin in September. The present results for October show a maximum only 0.1 that of Chandler. On the other hand, Chandler suggested that the species is absent during the winter, whereas appreciable numbers were present in the present study in January. The small numbers present in both October and January, as well as the low values reported by other authors for this very small species, support

the statement by Marsh (in Wright et al., MS, no date) that *T. prasinus* is "unimportant in ... Lake Erie."

All five of the regularly occurring diaptomids of Lake Erie were found in July, but in October only the two that had been the most common in July, namely *Diaptomus oregonensis* and *D. siciloides*, were encountered, and they were far from abundant. In January all five of the species again were present, and numbers were distinctly greater than in October (though far less than in July), despite the obvious recent mortality that had occurred. In January, *D. ashlandi* and *D. minutus* were the most common species. No ovigerous specimens of any species were seen, in contrast to a report by Davis (1961), who observed a great deal of breeding of *D. sicilis* in January of 1952.

In October, the recent calanoid invader of the Great Lakes, *Eurytemora affinis*, occurred, and ovigerous females were seen in all three basins. Adults were present only in minimal numbers, but juveniles of all copepodid stages from I to V were common at all of the Western Basin stations, with a maximum of $5.0 \times 10^3/m^3$ at station G9-1. This species was not seen in the January samples, and was encountered only at three stations in July.

DISCUSSION

Previous to the present study and the earlier one dealing with the July 1967 samples (Davis, 1968), there has been no good opportunity to examine the zooplankton of all three basins of Lake Erie, sampled by the same methods throughout, and analysed by the same techniques. All of the earlier quantitative investigations have been conducted in limited portions of one basin, and the different basins have been examined in different years by different investigators using differing techniques of collection and enumeration. (The investigations of Wilson (1929a, b, 1960) compared larger zooplankters from the Central and Eastern basins; although he reported his results in quantitative terms, his enumerations were based upon distinctly nonquantitative methods of collection; the studies of Burkholder (1929a, b, 1960) for planktonic rotifers and protozoans at the same stations in the Central and Eastern basins during the same period of time, were based on quantitative methods of collection, but were published only as generalizations.)

On the basis of the results obtained from the July 1967 samples it was concluded (Davis, 1968) that although the boundaries between the three basins of Lake Erie have been established more-or-less arbitrarily (especially the boundary between the Central and Eastern basins), and although there is a considerable overlap of communities among them, nevertheless there are distinct differences between them as reflected in the zooplankton populations. It was clear, however, and so stated, that generalizations based upon analysis of samples obtained in a single month could not safely be carried very far. The present results for October 1967 confirm the validity of the judgement that the three basins differ biologically. This is not strange, and has been hinted at before on the basis of the size of the fishery, general subjective estimates, etc., but there has been little hard evidence heretofore. The

TABLE 4. Numbers of Copepoda per m³ encountered during October 1967 (A, August

Species		Station	E2–1	F3–1	F3–2	G4–1	G6–1	G7–1	G9–1	G10–1	G12–1	E5–1	D9–1	D13–1	F8–1
Nauplii		A			6,340	13,600	13,800	21,700	11,200	22,600	15,400	15,200	15,600	23,700	21,400
,,		B	5,730	4,660	+	9,510		3,260		12,200	7,640	2,120	1,440	11,100	12,200
Juv. cyclopoids		A			900	3,400	1,130	1,740	3,260	6,880	11,800	16,900	6,670	8,150	13,580
,, ,,		B	640	6,550	1,080	2,310		1,250		2,350	1,870	2,450	500	1,810	2,040
Cyclops bicuspidatus	♀	A			+	+	+	+	+	+	+	+	+	150	3,600
,, ,,	♀	B	130	950	130	540		+		270	170	330	140	210	7,230
,, ,,	♂	A					+	+			360		+	440	4,640
,, ,,	♂	B	130	+	+	140		+		180	260	+	+	+	3,820
,, ,, ovig.		A													3,170
,, ,, ,,		B				+									+
C. vernalis	♀	A				+	+			+	+		+	+	+
,, ,,	♀	B	+	+	+	+									
,, ,,	♂	A							+	+	+		300	300	+
,, ,,	♂	B		+		+				+			+	+	
Mesocyclops edax	♀	A													+
,, ,,	♂	A									+				2,260
Tropocyclops prasinus	♀	A			+	410	+	+	310	180	+	430	+		
,, ,,	♀	B	+	+		270		+		270	170	+	70		
,, ,,	♂	A			+	+	250	220	+		+	+	+		
,, ,,	♂	B	+										+		
Canthocamptus robertcokeri	♀	A													+
,, ,,	♀	B				+				+					
,, ,, ♀, spermat.		A							+						
,, ,, juv.		B				+									
Diaptomus spp. juv.		A			+	+	+	+	200	610	+	+	150	520	1,850
,, ,, ,,		B		360				1,630			+		50	+	90
D. ashlandi	♀	B	+	1,190	130	540				1,180	+		+	+	
,, ,,	♂	B	+	600	190	480		110		540	170	+	+	+	
D. minutus	♀	B	130	480	640	410		1,630		450	420	650	+	210	
,, ,,	♂	B	+	360	450	270		110		360	+	+	100	+	
D. oregonensis	♀	A									+		+		160
,, ,,	♀	B	+	+	+	+		+		+	+	+	+	+	810
,, ,,	♂	A						+	+		+				+
,, ,,	♂	B		+	+			+		+	+		+		120
D. sicilis	♀	B		360		+		+		+				+	+
,, ,,	♂	B		+	+			+			+		+		
D. siciloides	♀	A									+	+	+		
,, ,,	♀	B								+					
,, ,,	♂	A					+							+	
,, ,,	♂	B	+										+	+	
,, ,, ovig.		A													
Epischura lacustris	♂	A											+		
Eurytemora affinis	♀	A						+					+	+	
,, ,,	♂	A								+			+	+	
,, ,, ovig.		A						+					+		
,, ,, juv.		A			180	1,900	1,120	870	4,990	1,000	820	2,810	1,180	300	

Western Basin is comparatively shallow (about 10 m) and to a considerable extent it is cut off from the Central Basin by the Lake Erie islands. Much of the water of the Western Basin is highly turbid, partly because of the silt load of some of the tributary rivers and partly because it is shallow with a broad expanse so that storms re-suspend bottom deposits. The Central Basin is less shallow, and its tributaries are much smaller; on the other hand it has

1967 (A ary 1968 (B). Blank = not encountered; + = seen, but in minimal numbers.

							Station											
D13-1	[illegible]8-1	H20-1	I23-1	J25-1	K28-1	L30-1	L33-1	L36-1	M38-1	M41-1	M43-1	N45-1	O47-1	P49-1	Q51-1	R53-1	S55-1	
3,700	21[illegible]400	7,420	12,400	17,800	16,900	28,700	33,500	37,600	43,800	14,300	11,600	14,400	14,600	21,300	14,600	22,400	10,600	
1,100	12[illegible]100																	
3,150	11[illegible]580	8,250	15,900	11,600	10,100	12,900	13,000	23,100	30,600	16,200	10,400	16,300	9,720	17,600	11,200	10,200	19,600	
1,810	2[illegible]040																	
150	[illegible]660	290	230	470	720		1,210	1,000	970	870	220	820	470	660	+	260	240	
210	7[illegible]230																	
440	[illegible]640	250	430	430	1,210		840	1,000	1,070	600	270	690	450	880	130	560	570	
+	[illegible]420																	
	[illegible]170	+	+	+	100	+	190	320	310	+	140	90	60	90			+	
	[illegible]																	
+	[illegible]				+													
	[illegible]																	
300	[illegible]																+	
+	[illegible]																	
	[illegible]	+	120	+	+	+	+	90		+	40	70	90					
	[illegible]260	+	+	190	150	900	90	180	150	140	270	90	170	90		+	+	
									+		60	40	30	250	+	200	+	
												+	+	130	210	260	+	
	[illegible]																	
	[illegible]	+	+															
	[illegible]																	
	[illegible]		+															
520	1[illegible]830	990	1,470	1,860	2,850	1,670	2,100	860	1,890	950	880	1,180	750	1,220	560	1,430	3,100	
+	[illegible]90																	
+	[illegible]																	
+	[illegible]																	
210	[illegible]																	
+	[illegible]																	
	[illegible]170		+	+	+	170	190	+	200	+	200	130	90	60	+	+	+	
+	[illegible]310																	
	[illegible]	+	+	80	150	390	330	+	310	+	100	170	110	+		100	+	
	[illegible]270																	
+	[illegible]																	
	[illegible]																	
	[illegible]			+		130		+	+	50	60	90	30	+		+		
+	[illegible]	+	+	80	+	+	+	+	+	50	+	40	+	130	+	+	+	
+	[illegible]																	
	[illegible]						+		+								+	
	[illegible]																	
+	[illegible]																	
+	[illegible]													+				
	[illegible]												+					
300	[illegible]															+		

even greater expanse. Effluents from large highly industrialized cities (Detroit, Toledo, Cleveland) empty into both the Western and Central basins, but the effect is more severe in the Western Basin because of the lesser volume of water and the partial isolation of the basin by the islands. The Eastern Basin is still deeper (the maximum off Long Point is 70 m) and the effluents that reach it are from comparatively small cities.

As in July, the October zooplankton of the Western Basin was characterized by a greater variety of protozoans and rotifers than the other basins. On the other hand, the number of protozoans in October was greater and the number of rotifers was less in the Western Basin than elsewhere, whereas in July these conditions were reversed. In October the biomass both of the cladocerans and of copepods was greatly reduced compared with that of July. Small cladocerans, however, were present in greater numbers than previously. The distribution of these varied among the three basins. *Bosmina coregoni* occurred mainly in the Eastern and Central basins, whereas *B. longirostris* was more abundant in the Western Basin and the shallow eastern portion of the Eastern Basin; it was insignificant at most Central Basin stations. *Chydorus sphaericus*, on the other hand, was relatively unimportant except in the Central Basin. Among the copepods the picture was similar. The Western Basin was very impoverished compared with the other basins, except that *Eurytemora affinis* (mostly juveniles) was almost completely confined there. Juvenile cyclopoids and immature diaptomid copepodid stages also were distinctly less common in the Western Basin, as were the adults of *Cyclops bicuspidatus*, *Mesocyclops edax*, and *Diaptomus oregonensis*. On the other hand, *Tropocyclops prasinus* was essentially lacking from the Central Basin, and the comparatively few specimens of *Cyclops vernalis* that were encountered were obtained from the Western Basin and some of the western-most stations of the Central Basin.

Unlike the situation in July, it appears that the zooplankton biomass did not decrease from west to east in October as it theoretically should judging from other indications and from common belief. The shallow Western Basin, on criteria suggested by Rawson (1955) and others, should be more productive than the Central Basin, and the Eastern Basin should be the least productive of the three. Most studies, including the results from the July 1967 cruise in the present series, support this hypothesis. One must not, however, judge any ecosystem by a partial analysis (e.g., of the zooplankton alone) during a single period of its existence. For example, from the same stations, occupied at the same dates in October, Kleveno (personal communication) showed that the ratios of total seston, chlorophyll *a*, and phytoplankton in the Central and Eastern basins, compared with the Western Basin are as shown in Table 5. This table indicates that all three of these parameters show the expected decrease from west to east, and hence the discrepancy in the distribution of the zooplankton perhaps was a temporary phenomenon, caused by some unrecorded environmental change over the period preceding sampling.

TABLE 5. Ratios of seston, chlorophyll *a*, and phytoplankton in the Western Basin (W) compared with the Central Basin (C) and the Eastern Basin (E) during October 1967. Calculated from Kleveno (personal communication).

Seston		Chlorophyll *a*		Phytoplankton	
W:C	W:E	W:C	W:E	W:C	W:E
3.2	7.5	3.2	4.7	5.1	15.3

The timing of the January cruise was unfortunate in that the very severe (subzero F) weather prevented visiting all three basins, even with an icebreaker, but it was of great interest for the marked mortality (winterkill?) of microcrustaceans that occurred. The distortion of a large proportion of the decayed specimens prevented an accurate count, so that no sound estimate of the proportion destroyed could be obtained but it appeared that considerably more than half had been killed. It would have been of interest to know the rate of the pre-kill fall of water temperatures.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the kindness of Mr Conrad O. Kleveno of U.S. F.W.P.C.A., who made the collected samples available to me, and the generosity of Mr George L. Harlow, Director, Cleveland Program Office of U.S. F.W.P.C.A., who gave permission to publish the data. Thanks also are offered to Case Western Reserve University, Cleveland, Ohio, where most of the analyses of the samples were performed.

REFERENCES

AHLSTROM, ELBERT H. 1933. A quantitative study of Rotatoria in Terwilliger's Pond, Put-in-Bay, Ohio. Ohio State Univ. Bull. 38: 1–36.

ANDREWS, THEODORE F. 1949. The life history, distribution, growth, and abundance of *Leptodora kindtii* (Focke) in western Lake Erie. Abstr. Doctoral Dissertations Ohio State Univ. 57: 5–11.

1953. Seasonal variations in relative abundance of *Cyclops vernalis* Fischer, *Cyclops bicuspidatus* Claus, and *Mesocyclops leuckarti* (Claus) in western Lake Erie, from July, 1946, to May, 1948. Ohio J. Sci. 53: 91–100.

BEETON, ALFRED M. 1966. Indices of Great Lakes eutrophication. Great Lakes Res. Div., Univ. Mich. Publ. 15: 1–8.

BROOKS, JOHN L. 1959. Cladocera, p. 587–656. *In* W. T. Edmondson [ed.] Fresh-water biology. John Wiley & Sons, Inc., New York.

BURKHOLDER, PAUL C. 1929a. Microplankton studies in Lake Erie. Bull. Buffalo Soc. Nat. Sci. 14: 73–93.

1929b. Microplankton studies in Lake Erie. New York State Conserv. Dept., Suppl. Ann. Rept. 18: 60–66.

1960. A survey of the microplankton of Lake Erie. U.S. Fish Wildlife Serv., Spec. Sci. Rept. Fish. 334: 123–144.

CHANDLER, DAVID C. 1940. Limnological studies of western Lake Erie. I. Plankton and certain physical-chemical data of the Bass Islands region, from September, 1938 to November, 1939. Ohio J. Sci. 40: 291–336.

DAVIS, CHARLES C. 1954. A preliminary study of the plankton of the Cleveland Harbor area, Ohio. III. The zooplankton and general ecological considerations of phytoplankton and zooplankton production. Ohio J. Sci. 54: 388–408.

1961. Breeding of calanoid copepods in Lake Erie. Verhandl. Intern. Ver. Theoret. Angew. Limnol. 14: 933–942.

1962. The plankton of the Cleveland Harbor area of Lake Erie in 1956–57. Ecol. Monographs 32: 209–247.

1966. Plankton studies in the largest great lakes of the world, with special reference to the St. Lawrence Great Lakes of North America. Great Lakes Res. Div., Univ. Mich. Publ. 14: 1–36.

1968. The July 1967 zooplankton of Lake Erie. Proc. Conf. Great Lakes Res. 11: 61–75.

NIPKOV, FRITZ. 1956. *Epistylis rotans*, Švec im Plankton des Zürichsees. Schweiz. Z. Hydrol. 18: 161–170.

PENNAK, ROBERT W. 1953. Freshwater invertebrates of the United States. Ronald Press, New York. ix + 769 p.

RAWSON, DONALD S. 1955. Morphometry as a dominant factor in the productivity of large lakes. Verhandl. Intern. Ver. Theoret. Angew. Limnol. 12: 164–175.

RYLOV, W. M. 1935. Das Zooplankton der Binnengewässer. Die Binnengewässer 14: i–xi, 1–272.

VORCE, C. M. 1882. Microscopical forms observed in the water of Lake Erie. Proc. Am. Soc. Microscop. 5: 187–196.

WILSON, CHARLES B. 1929a. The macroplankton of Lake Erie. Bull. Buffalo Soc. Nat. Sci. 14: 94–135.

1929b. The macroplankton of Lake Erie. New York State Conserv. Dept., Suppl. Ann. Rept. 18: 67–76.

1960. The macroplankton of Lake Erie. U.S. Fish Wildlife Serv., Spec. Sci. Rept. Fish. 334: 145–172.

WRIGHT, STILLMAN, L. H. TIFFANY, AND W. M. TIDD. 1955. Limnological survey of western Lake Erie. U.S. Fish Wildlife Serv., Spec. Sci. Rept. Fish. 139: 1–341.

MS, No date. Supplementary data to a limnological survey of western Lake Erie. Ohio Div. Wildlife. 288 p. (Mimeo.)

Effect of Tidal Barriers Upon the M_2 Tide in the Bay of Fundy

KENNETH B. YUEN

Marine Sciences Branch
Department of Energy, Mines and Resources
Ottawa, Ont.

YUEN, KENNETH B. 1969. Effect of tidal barriers upon the M_2 tide in the Bay of Fundy. J. Fish. Res. Bd. Canada 26: 2477–2492.

A model was constructed for the M_2 tide in the Bay of Fundy, in which the two-dimensional hydrodynamical equations with quadratic friction were integrated in time. After calibration tests, in which the normal tide was reproduced, calculations were carried out to determine any changes from the normal configuration caused by the presence of tidal barriers in the vicinity of Cape Enrage, Cape Sharpe, and Cape Chignecto.

The effect in each instance was a reduction of amplitude and an advancement of the time of high water. The maximum amplitude reductions were as follows: single barrier at Cape Sharpe, 15%; single barrier at Cape Enrage, 13%; both barriers at Cape Sharpe and Cape Enrage, 19 and 20%; twin barrier at Cape Chignecto, 21–27%.

Received March 19, 1969.

INTRODUCTION

THE BAY OF FUNDY (Fig. 1) has attracted the attention of the mariner, scientist, and curious onlooker for many years because of its remarkable tides. For example, at Burncoat Head, the mean spring range is 38.4 ft, but heights of well over 50 ft have been recorded (see Dawson, 1917). It is not surprising that many scientific studies have been conducted in this area. In view of such a large tidal range, the possibility of economical extraction of power from the Bay of Fundy tides cannot be ignored. Attention has already been focused upon the Passamaquoddy Bay area (e.g., International Passamaquoddy Fisheries Board, 1959; International Passamaquoddy Engineering Board, 1961), although interest in that area has died down somewhat in recent years. Presently, the feasibility of tidal power development in the upper reaches of the Bay of Fundy is under intensive study by the Atlantic Tidal Power Programming Board. If the presence of a tidal barrier were to greatly reduce the tidal amplitude, then the extraction of power might no longer be economical. The objective of this work is to determine the effects that the presence of a tidal barrier (or barriers) would have on the present tidal regime.

Such a study of the Bay of Fundy tides may, for simplicity, be restricted to the M_2 constituent, although ideally all the important constituents should be considered. In the Bay of Fundy, it is found that the semi-diurnal tide represents over 92% of the total regime. Furthermore, it is found that the

Printed in Canada (J1421)

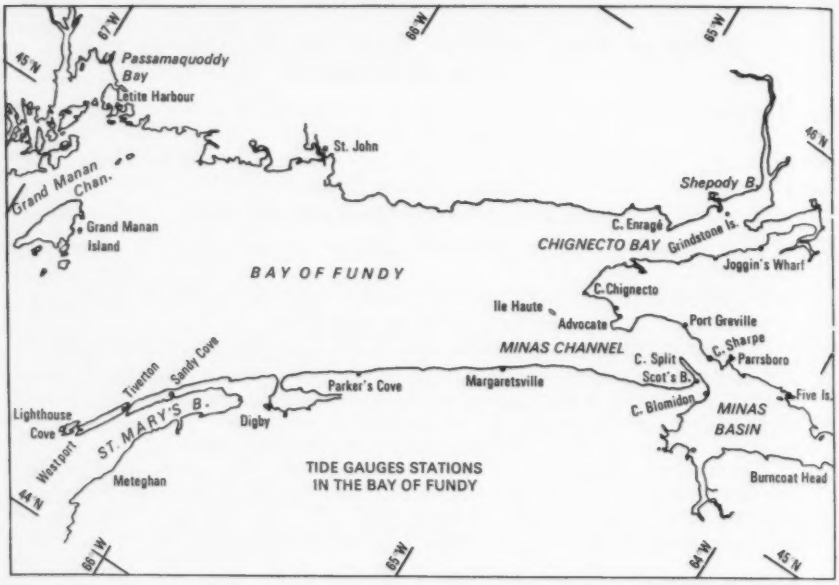

FIG. 1. The Bay of Fundy, including the location of tide gauge sites from which data were utilized for this study.

M_2 constituent represents about 86% of the mean spring range of the semi-diurnal tide in this area. It seems, therefore, reasonable to restrict this study to the M_2 constituent alone.

THE BAY OF FUNDY M_2 TIDE

The co-amplitude and co-phase (cotidal) surfaces for the observed M_2 tide are shown in Fig. 2. These lines are an interpretation by the author. It is apparent that a study of the tidal regime must necessarily incorporate a two- rather than a one-dimensional approach, although one-dimensional calculations were carried out in preliminary investigations. In particular, note that the direction of propagation of the incident wave in the outer regions of the bay is not in the longitudinal direction. Langford's (MS, 1966) interpretation has the cotidal lines oriented even more obliquely to the longitudinal axis of the bay. This has been substantiated by the existence of transverse currents in the outer regions of the bay (see Langford, MS, 1966; Anon., 1959).

Contrary to popular misbeliefs, the large tides in the Bay of Fundy are not due to resonance of the M_2 constituent. Recently Rao (1968) calculated the natural period of the lowest longitudinal mode of the bay to be 9.047 hr, as compared with the M_2 period of 12.42 hr. The indication is that the Bay of Fundy is too short for a high degree of resonance of the M_2 tide to occur.

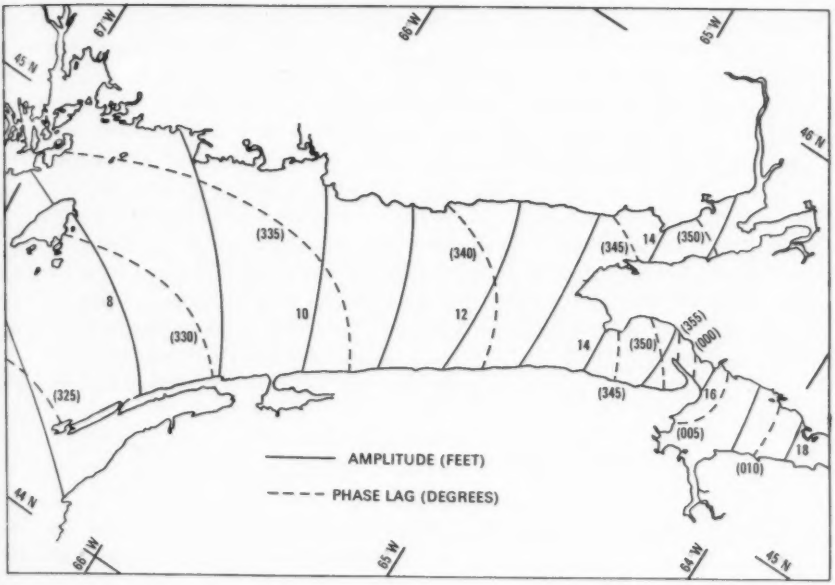

FIG. 2. The observed M_2 tide, based on coastal data; the solid line represents the amplitude, the broken line the phase lag.

In an examination of tidal amplification between Lighthouse Cove and Five Islands, it was found that the semi-diurnal constituents were amplified by about 2.50. For the diurnal constituents, this value is less than 1.50, so that a small degree of resonance does indeed occur in the M_2 tide.

What are the effects of a barrier expected to be? The natural period of the bay is already shorter than the M_2 period, and would be further shortened by the erection of a barrier. Thus, generally speaking, a barrier would cause a reduction in the tidal amplitude. The incoming tidal wave would also be reflected at the barrier, resulting in the advancement of the time of high water at points on the seaward side of the barrier.

In the region bounded by the upper reaches of the bay and by the seaward boundary of the grid system shown in Fig. 3, the potential energy of the M_2 is estimated to be 4.1×10^{14} ft-lb, or 154 million kw-hr. This energy is distributed as follows: main part of the Bay of Fundy, 67.1%; Chignecto Bay, 9.5%; Minas Channel, 5.6%; and Minas Basin, 18.0%. The potential of the Minas system is 2.5 times that of the Chignecto system. Even if Minas Channel were ignored, this ratio is still 1.9, so that a site in Minas Channel would permit greater extraction of energy. The values represent the maximum amount obtainable in a power project only if the reservoir could be suddenly and completely emptied at high water. In reality, the reservoir can only be emptied at a finite rate depending on the turbine capacities and the head

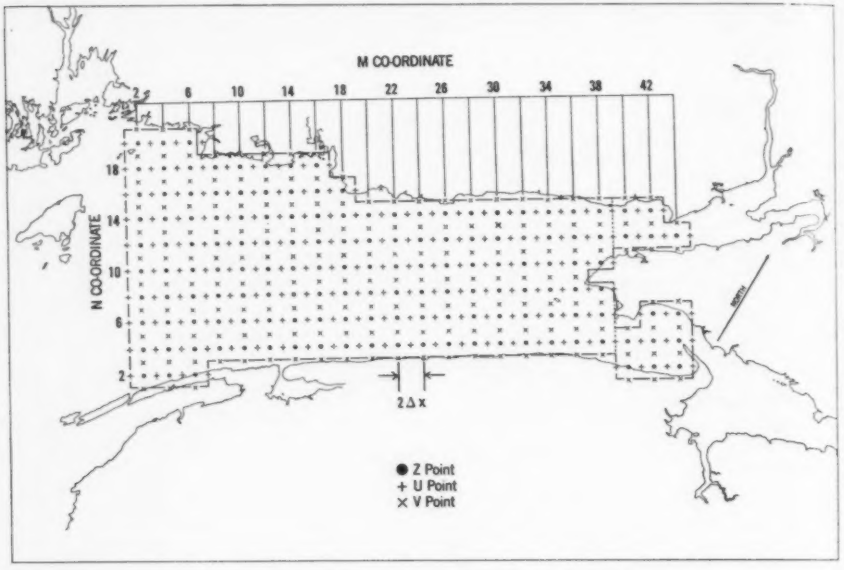

FIG. 3. Grid system used for the Bay of Fundy calculations.

difference between the two sides of the barrier. Furthermore, immediately after high water, the water level on the seaward side of the barrier is increasing. Therefore, the practical amount of energy that could be extracted will be much less than the figures quoted here. In any event, when a barrier is constructed, the energy that is normally dissipated upstream of the barrier site must then be re-distributed downstream or seaward of the site. One would therefore expect a barrier in Minas Channel to create greater changes in tidal configuration than would a barrier in Chignecto Bay.

HYDRODYNAMICAL EQUATIONS

The vertically integrated hydrodynamical equations in rectangular coordinates for a homogeneous body of water are given by

$$\frac{\partial U}{\partial t} = - r(U^2 + V^2)^{\frac{1}{2}} \frac{U}{H} + fV - g \frac{\partial \zeta}{\partial x} \tag{1}$$

$$\frac{\partial V}{\partial t} = - r(U^2 + V^2)^{\frac{1}{2}} \frac{V}{H} - fU - g \frac{\partial \zeta}{\partial y} \tag{2}$$

$$\frac{\partial \zeta}{\partial t} = - \frac{\partial}{\partial x}(HU) - \frac{\partial}{\partial y}(HV) \tag{3}$$

where ζ = height of water surface above equilibrium level; U, V = components of the depth mean velocities in the x- and y- directions, respectively; H = total depth of the water column; g = acceleration of gravity; f = Coriolis parameter, = $2\,\Omega \sin \phi$; Ω = angular speed of rotation of the Earth; ϕ = latitude, North; and r = coefficient of quadratic friction.

These equations are subject to the following assumptions: (1) the length scale of the motion is large compared with the depth, so that the vertical velocity is negligible and the pressure may be expressed by the hydrostatic approximation; (2) the velocities are small, so that the nonlinear accelerative terms may be neglected; (3) the Coriolis parameter, f, is taken constant over the whole of the basin; and (4) only the bottom stresses are considered, and these are assumed to be expressible in the quadratic form used above.

To facilitate the numerical integration of equations 1–3, a central difference approximation was used for the derivatives, as

$$\frac{\partial}{\partial s} F(s) = \frac{F(s + \Delta s) - F(s - \Delta s)}{2\Delta s} + O\left[\frac{(\Delta s)^2}{3!} \frac{\partial^3 F(s)}{\partial s^3}\right] \tag{4}$$

where F represents U, V, or Z, and s represents x, y, or t. The following notation will be used in the discussions below:

$$F^t_{m,n} = F(y = m\Delta y, x = n\Delta x, t' = t\Delta T) \tag{5}$$

where m, n, and t are taken to be integers. The grid system used for the calculations is shown in Fig. 3, in which all similar points are 2 grid lengths apart on a square grid; that is,

$$\Delta x = \Delta y. \tag{6}$$

For convenience, now let m, n, and t' represent even integers only. The evaluation of the left-hand side of equation 1 at even time steps requires

$$\frac{\partial U^t}{\partial t} = \frac{U^{t+1} - U^{t-1}}{2\Delta t} \tag{7}$$

and similarly for V in equation 2. Thus, values of U and V are calculated for odd time steps. For equation 3, the evaluation of the left-hand side at odd time steps requires

$$\frac{\partial \zeta^{t+1}}{\partial t} = \frac{\zeta^{t+2} - \zeta^t}{2\Delta t} \tag{8}$$

so that ζ is calculated for even time steps. With the use of equations 7 and 8, equations 1–3 may now be integrated by the "leap frog" technique, providing approximations are found for all the terms on the right-hand side of these equations.

In the equations of motion, 1 and 2, the Coriolis terms require a knowledge of V at the U-points, and vice versa, in which instance the following spatial averages are used:

$$U_{m+1,n} = \tfrac{1}{4}\left[U_{m+2,n-1} + U_{m,n-1} + U_{m+2,n+1} + U_{m,n+1}\right] \tag{9}$$

$$V_{m,n+1} = \tfrac{1}{4}\left[V_{m+1,n} + V_{m-1,n} + V_{m+1,n+2} + V_{m-1,n+2}\right]. \tag{10}$$

The velocities in equations 9 and 10 are required at even time steps, but these are not known so that the values at the previous (odd) time steps are used in obtaining the Coriolis accelerations.

With D defined as the equilibrium depth, the total depths are approximated as

$$H_{m+1,n} = D_{m+1,n} + \tfrac{1}{2}\left[\zeta_{m+2,n} + \zeta_{m,n}\right] \tag{11}$$

$$H_{m,n+1} = D_{m,n+1} + \tfrac{1}{2}\left[\zeta_{m,n+2} + \zeta_{m,n}\right]. \tag{12}$$

Briefly then, the difference equations are

$$\zeta^{t+2}_{m,n} = \bar{\zeta}^{t}_{m,n} - \frac{\Delta t}{\Delta x}\left[\begin{array}{l} H^{t+1}_{m,n+1}\, U^{t+1}_{m,n+1} - H^{t+1}_{m,n-1}\, U^{t+1}_{m,n-1} \\ + H^{t+1}_{m+1,n}\, V^{t+1}_{m+1,n} - H^{t+1}_{m-1,n}\, V^{t+1}_{m-1,n} \end{array}\right] \tag{13}$$

$$\begin{aligned} U^{t+1}_{m,n+1} = \bar{U}^{t-1}_{m,n+1}&\left[1 - \frac{2\Delta t r\left\{\left[U^{t-1}_{m,n+1}\right]^2 + \left[V^{t-1}_{m,n+1}\right]^2\right\}^{\frac{1}{2}}}{H^{t}_{m,n+1}}\right] \\ &+ 2\Delta t f V^{t-1}_{m,n+1} - \frac{\Delta t}{\Delta x}\, g\left[\zeta^{t}_{m,n+2} - \zeta^{t}_{m,n}\right] \end{aligned} \tag{14}$$

$$\begin{aligned} V^{t+1}_{m+1,n} = \bar{V}^{t-1}_{m+1,n}&\left[1 - \frac{2\Delta t r\left\{\left[U^{t-1}_{m+1,n}\right]^2 + \left[V^{t-1}_{m+1,n}\right]^2\right\}^{\frac{1}{2}}}{H^{t}_{m+1,n}}\right] \\ &- 2\Delta t f U^{t-1}_{m+1,n} - \frac{\Delta t}{\Delta x}\, g\left[\zeta^{t}_{m+2,n} - \zeta^{t}_{m,n}\right]. \end{aligned} \tag{15}$$

In each of the above equations, the horizontal bar over the first term on right-hand side denotes a smoothing process carried out after each integration to reduce numerical instability. For example,

$$\bar{\zeta}_{m,n} = \sigma\zeta_{m,n} + \frac{(1-\sigma)}{4}\left[\begin{array}{l} \zeta_{m+2,n} + \zeta_{m-2,n} \\ + \zeta_{m,n+2} + \zeta_{m,n-2} \end{array}\right] \tag{16}$$

where σ is a stability factor, with a value between 0 and 1. Equation 16 can be reduced to the form

$$\bar{\zeta}_{m,n} = \zeta_{m,n} + A\nabla^2\zeta_{m,n} \tag{17}$$

where ∇^2 is the Laplacian operator, and A is similar to a coefficient of lateral diffusion of momentum (see Fischer, 1965).

Miyazaki (1965) and Brettschneider (1963) have given the criterion for numerical stability of this system of difference equations as

$$\Delta t < \frac{\Delta x}{\sqrt{2gH_{max}}}. \tag{18}$$

In a system of this sort, spurious waves may develop, the most bothersome of which is generally those of wavelength $4\Delta 1$ (Harris and Jelesnianski, 1964). Equation 18 ensures that the period of the gravitational oscillations under study is longer than the period of these particular spurious waves.

BAY OF FUNDY MODEL

The actual grid system used to approximate the Bay of Fundy is shown in Fig. 3, where $2\Delta 1$ has been set to 25,760 ft. The value of $2\Delta t$ has then been taken to be 120 sec, which satisfies the stability criterion in equation 18. Several shortcomings of the model are apparent. Firstly the approximation of the real shoreline is limited to integral multiples of the double grid length. In particular, this does not permit good approximation of Minas Channel. Secondly, the seaward boundary of the grid extends only to the vicinity of Passamaquoddy Bay. A much better, but still practical boundary would have been the Cutler–Yarmouth line used by Rao (1968) and Greenberg (1969). Ideally, it may be necessary to extend the model to include the whole of the Gulf of Maine also, but the astronomical increase in the number of calculations would make this rather impractical. The shortcomings of the present boundary are discussed further in case E, below.

Along the shorelines, the normal component of the velocities are all set to zero. Along the open boundary ($n = 2$), the vertical displacement is given by the observed M_2, as

$$\zeta = A\cos(wt - g) \tag{19}$$

where g is the phase lag of the M_2 constituent. In each calculation it was assumed that the presence of a tidal barrier did not seriously affect the tide at the outer boundary.

Note in the discussion below that ζ and Z represent the same variable; Z was used in the computer programming.

Five calculations will be presented, as follows:

Case	Cape Chignecto	Cape Enrage	Cape Sharpe
A	–	Z observed	Z observed
B	–	Z observed	Barrier
C	–	Barrier	Z observed
D	–	Barrier	Barrier
E	Barrier	–	–

TIDAL BARRIERS

Case A. Normal M_2 Configuration

The values of σ, the stability factor, and r, the friction coefficient, were adjusted during trial calculations for the normal M_2 regime. In this instance, the observed M_2 tide was input at the points Z(4,46) and Z(12,44). After a series of tests, it was found that the use of $\sigma = 0.99$ and $r = 0.003$ gave the best results. Over most of the model, the calculated M_2 tide (see Fig. 4) differed from the observed tide by less than 0.2 ft in amplitude and 2° in phase lag. In Minas Channel, the discrepancies are slightly larger.

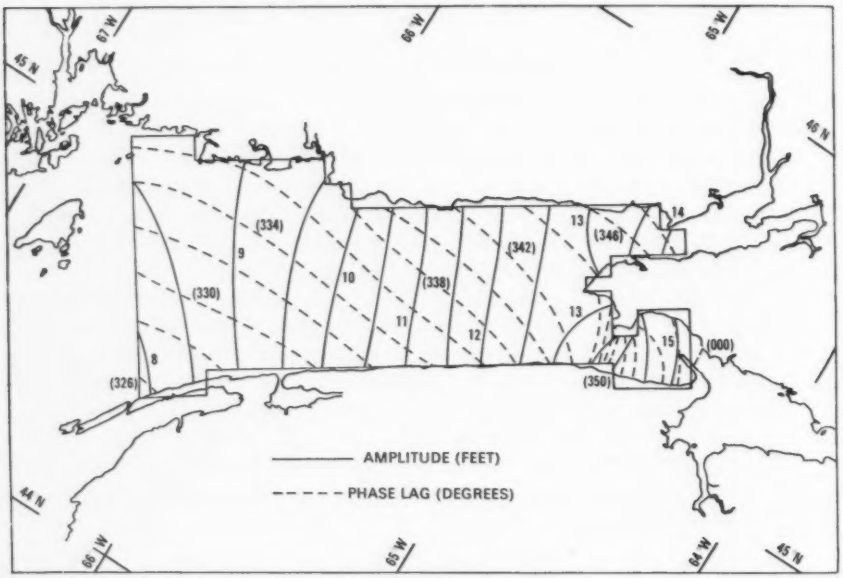

FIG. 4. Calculated normal M_2 tide; the solid line represents the amplitude, the broken line the phase lag, case A.

Two main factors contribute to the discrepancies in Minas Channel. Firstly, the mouth in the model is much narrower than the mouth of the real channel, a weakness that had already been mentioned. Note the crowding of the cotidal lines there. Secondly, the "observed" tide is only an interpretation based on the analyses of data that were obtained from shore-based tide gauge stations. Furthermore, the lengths of record of these data were in many instances only 1 month, so that there are possible errors in the values of the analyzed constituents. In any case, we are no longer studying the Bay of Fundy, but an approximate model of the Bay of Fundy. Thus, the results of case A will be used as the standard for the observed M_2 tide.

Case B. Single Barrier at Cape Sharpe

A complete barrier was erected in the vicinity of Cape Sharpe by setting U(4,45) equal to zero. It is (dubiously) assumed that the presence of this barrier will have no significant effect on the tide at Z(12,44), where the tide is taken as the observed M_2. The effect of the barrier upon the normal configuration is conveniently measured in terms of a percentage decrease in tidal amplitude (Fig. 5). As expected from earlier discussions, there is a general

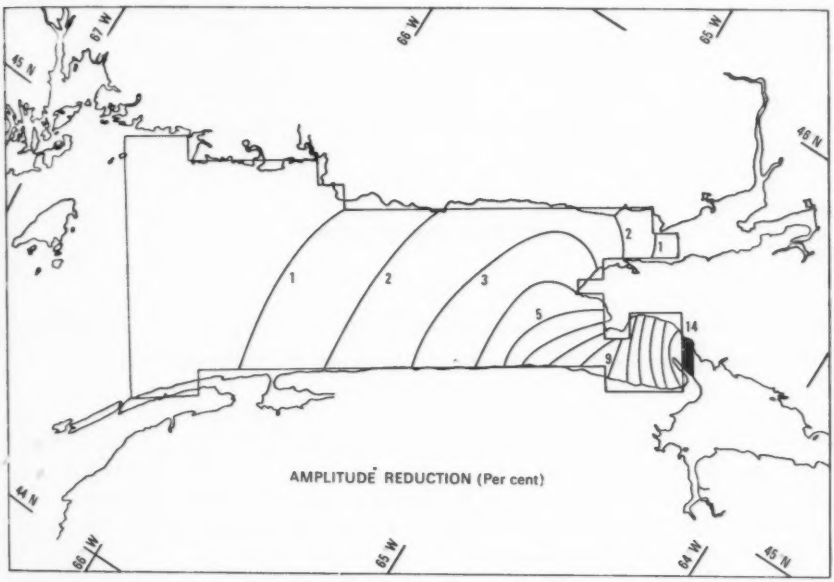

Fig. 5. Percentage decrease in M_2 amplitude due to barrier at Cape Sharpe, case B.

decrease in amplitude. The Bay of Fundy is already too short for the M_2 oscillation to be resonant, and thus a shortening of the bay (the erection of a barrier) must decrease the tidal amplitude. Most of the perturbation is confined to the Minas Channel region, with a maximum decrease of 15% at the barrier site. This is in rough agreement with the value of 20% obtained from two preliminary one-dimensional models, one an analytical model, and the other a dynamical model. However, the one-dimensional models cannot take into account the transverse flows near the mouth of the bay nor those near Cape Chignecto and in Minas Channel (see Yuen, MS, 1967).

The decrease in phase lag (Fig. 6) is attenuated even more quickly than the effect on the amplitude. A decrease in phase lag is equivalent to an advance in the time on high water. This is certainly reasonable, since the incident tidal wave is now reflected at the barrier rather than propagating to the upper

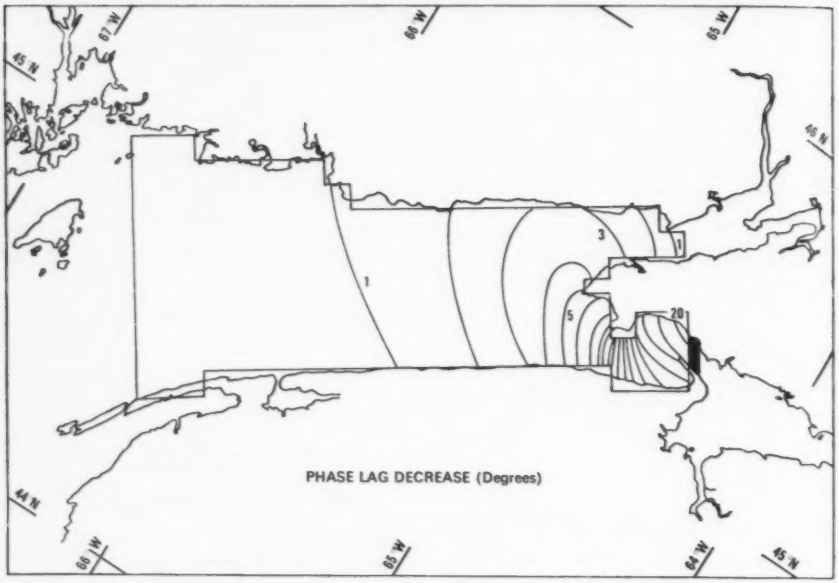

FIG. 6. Decrease in M_2 phase lag due to barrier at Cape Sharpe, case B.

reaches of the estuary where it would normally be partially reflected and partially dissipated by bottom stresses. A maximum decrease of 20° is found at the barrier site. This is greater than the value of 12° from the one-dimensional dynamical calculations, but it is suspected that the friction term in that preliminary model was too low.

From Fig. 5 and 6, most of the changes from the normal tidal regime are restricted to the area near the barrier. This indicates that the assumption that the barrier has no effect upon the tide at the mouth of the model is valid for this case (B).

In the normal M_2 regime, the largest value of $u\partial u/\partial x$ was found to be about 4×10^{-4} ft sec $^{-2}$ at the mouth of Minas Channel, which is much less than $\partial u/\partial t$ for most of the tidal cycle. For this calculation, the largest value was about 4×10^{-6} ft sec $^{-2}$. A calculation with this nonlinear term included showed no significant difference in the results from those obtained with linear equations. Thus, the neglect of the nonlinear $u\partial u/\partial x$ term is quite valid.

CASE C. SINGLE BARRIER NEAR CAPE ENRAGE

For this barrier, the value of U(12,45) was set to zero. It is also assumed that the effect on this barrier upon Z(4,46) is small, and from the results this appears to be a safe assumption. Again, the largest effects are found at the barrier site (Fig. 7 and 8), with a 13% decrease in amplitude and a 5° retardation

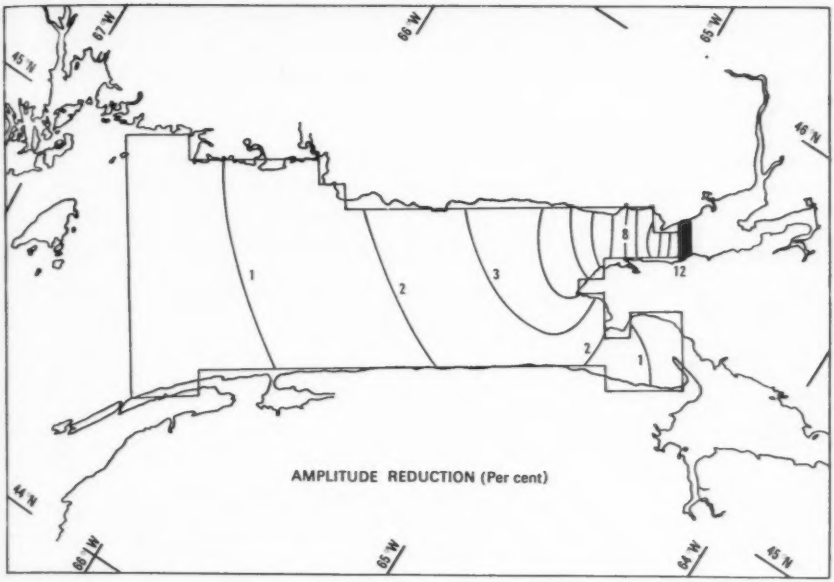

FIG. 7. Percentage decrease in M_2 amplitude due to barrier at Cape Enrage, case C.

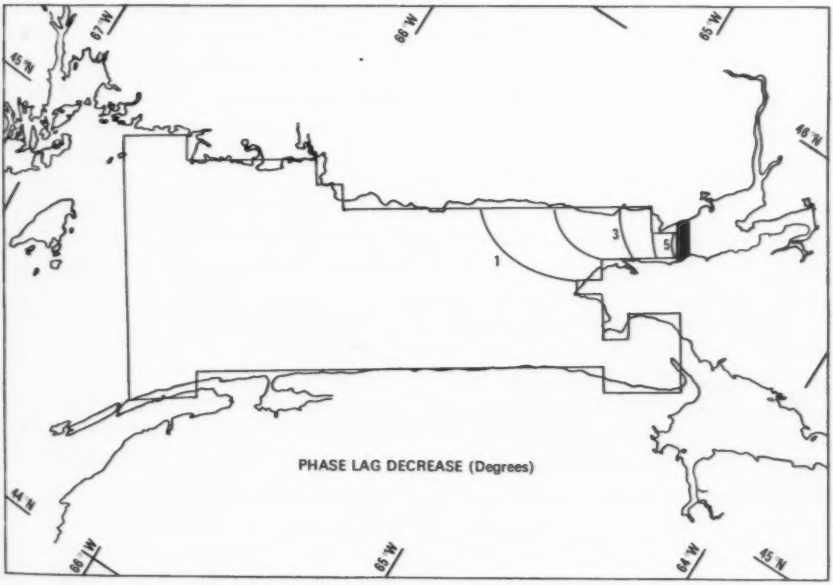

FIG. 8. Decrease in M_2 phase lag due to barrier at Cape Enrage, case C.

in the phase lag. From this case and case B, it is apparent that Minas Channel plays a much larger role in the tidal hydraulics of the Bay of Fundy system than does the Chignecto Bay system, as was indicated by the distribution of potential energy.

Case D. Two Barriers, Cape Sharpe and Cape Enrage

For the twin barriers, both U(4,45) and U(12,45) were set to zero. The decreases in amplitude (Fig. 9) are much larger than in cases B and C, now 19 and 20% at the Cape Enrage and Cape Sharpe barrier sites, respectively,

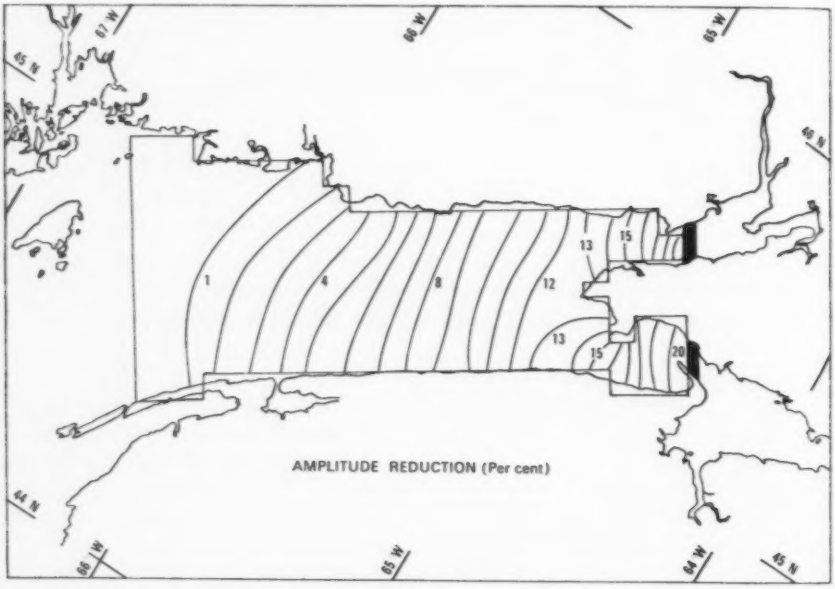

Fig. 9. Percentage decrease in M_2 amplitude due to barriers at Cape Sharpe and Cape Enrage, case D.

as compared with values of 13 and 15% respectively, for each of the barriers alone. The maximum decreases in phase lag (Fig. 10) are 13° and 24° at the barrier sites, respectively, as compared with values of 5 and 20° for each barrier alone. This indicates that each barrier has some effect upon the tide at the other barrier site, and that the two barriers in combination produce effects greater than the sum of the effects when each is considered exclusively of the other.

Case E. Two Barriers near Cape Chignecto

In this final calculation, the two barriers of case D were shifted seaward to the points U(4,39) and U(12,39). It is quite probable that the effect of

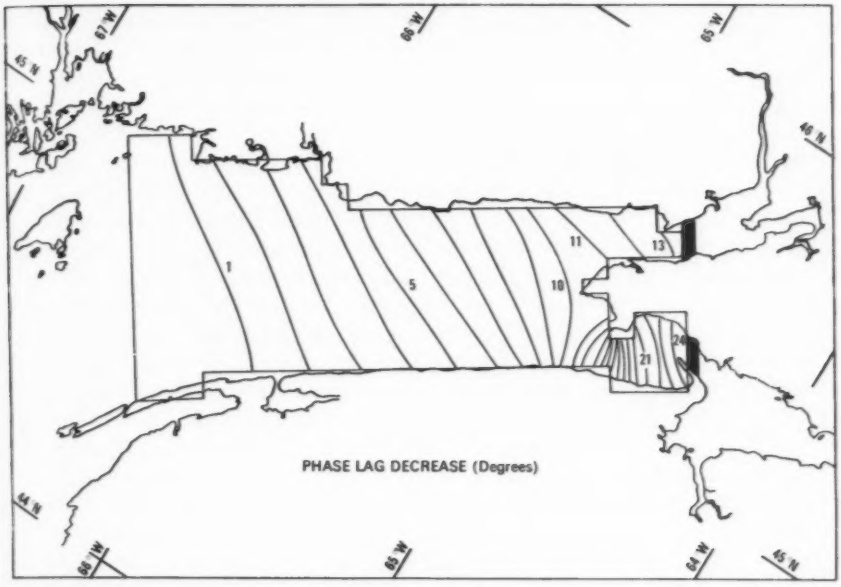

FIG. 10. Decrease in M_2 phase lag due to barriers at Cape Sharpe and Cape Enrage, case D.

these barriers cannot be neglected at the outer boundary. Nevertheless, this case is presented mainly to provide a rough estimate of the change in the barrier effect as the position of the barrier is shifted from that in case D to its present position. In actual fact, case E is not economically important since the Atlantic Tidal Power Programming Board is now concentrating much of its studies on the Shepody Bay–Cumberland Basin area, rather than locations further seaward, such as Cape Sharpe.

The reductions (Fig. 11) at the barrier sites were found to be 21% and 27% in Chignecto Bay and Minas Channel, respectively. In case D, the corresponding values were 19% and 20%. The 7% change in Minas Channel, compared with the 2% change in Chignecto Bay again confirms the fact that the Minas Bay branch plays a much more important role in the Bay of Fundy tidal system than does the Chignecto Bay branch.

Several other authors have carried out studies, with improved seaward boundary positions. G. F. D. Duff (personal communication) constructed a one-dimensional frictionless model, assuming sinusoidal time dependence for the solutions. By taking his outer boundary at the same position as in this study, he obtained a 23% reduction in amplitude for a barrier similar to my case E, which is the one case in the present study where the position of the seaward boundary is questionable. Greenberg (1969) also performed some calculations, a one-dimensional model with linear friction. He chose his outer boundary along a line joining Cutler, Maine and Yarmouth, Nova Scotia. This is identical

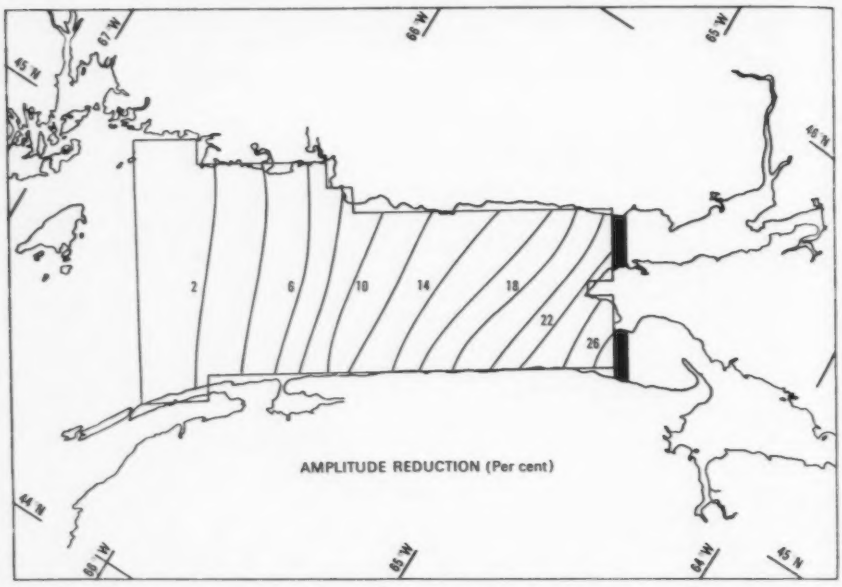

FIG. 11. Percentage decrease in M_2 amplitude due to two barriers at Cape Chignecto, case E.

to Rao's (1968) boundary, and is a very reasonable boundary for the Bay of Fundy proper. Greenberg obtained a reduction of 28% for a Cape Chignecto barrier. Duff also shifted his seaward boundary to coincide with that of Greenberg and obtained the same result. On this basis, my results of 21 and 27% are in rough agreement with those of Greenberg and Duff; the neglect of the effect at the seaward boundary seems to have made only a few percent difference.

It must be pointed out that Duff extended his seaward boundary further. In fact, he extended it "to infinity" and arrived at a rather high figure of 37%. The point of Duff is in principle a good one. Unfortunately, beyond the Cutler–Yarmouth line, Duff's schematization of the Bay of Fundy and Gulf of Maine system is not an accurate one. Indeed, two-dimensionality cannot be ignored. At the same time, over such an extent, phase changes cannot be ignored either, although up to the Cutler–Yarmouth line Duff's neglect of phase changes is of no serious consequence. The usefulness of Duff's work is that it shows the changes in the results as the seaward boundary is changed from the position in the present (Yuen's, MS, 1967) model to that of Greenberg (1969). A comparison of Yuen's and Greenberg's results would not necessarily show this, but rather may be indicative of differences in the models themselves.

CONCLUSIONS

The effect of tidal barriers upon the normal M_2 tidal configuration has been studied through the use of a two-dimensional numerical model, involving

time integration of the hydrodynamical equations. Four barrier cases were discussed: a single barrier at Cape Sharpe; a single barrier at Cape Enrage; both these barriers simultaneously; and a double barrier near Cape Chignecto. In general, a decrease in tidal amplitude and an advancement of the time of high water resulted from the presence of the barriers.

For the various barriers, the following maximum decreases were found in the tidal amplitude at the barrier site: single barrier at Cape Sharpe, 15%; single barrier at Cape Enrage, 13%; both barriers at Cape Sharpe and Cape Enrage, 19 and 20%; and twin barrier at Cape Chignecto, 21–27%.

For each case, it was assumed that the tide at the seaward boundary was not affected by the presence of the barrier, but the results indicated that this was not true for the Cape Chignecto twin barrier. Duff (personal communication) and Greenberg (1969) have both investigated this case, but with one-dimensional models. Greenberg found a value of 28% and Duff a value of 37%. However, the author feels that although the point is well taken, Duff's results may be slightly over-estimated. A more reasonable value probably lies between 27 and 37%. Fortunately, the case in question is not an important one as far as economical barrier sites is concerned, and in the other more important cases, the position of the seaward boundary is not so dubious.

For the more feasible sites (cases B, C, and D) the amplitude reduction is at most 20%, which on the surface would not appear to be a serious reduction. The M_2 represents only about 86% of the Bay of Fundy tide, and since the reductions in the other constituents are at worse about the same and probably less, the reduction of the M_2 tide is a reasonable upper limit for the reduction of the complete tide. These reductions are for complete barriers only. In a real tidal power development, there is effectively only a partial barrier. The greater the flow that is permitted to pass through the barrier, the smaller would be the tidal reduction. In effect, this can only be determined by the number, size, and capacity of the turbines. A useful extension of this work would be to calculate the effects due to partial barriers, by setting the flow equal to various fractions of the normal unperturbed tidal streams. In effect, the results given here are only conservative upper limits to what may result from an actual power project.

Even though larger decreases were found for barriers in Minas Channel than for barriers in Chignecto Bay, the tidal amplitudes still remained larger in Minas Channel. It was shown that the energy potential of the Minas Channel branch is also larger than for Chignecto Bay, and would appear to be still true when a barrier is erected. Thus, a site in Minas Channel would appear to be more desirable than one in Chignecto Bay.

Other considerations may be more important, though, in the choice of the site(s). It is possible that the potential of the various regions of the Bay of Fundy so exceeds power requirements to the extent that the predicted barrier effects may no longer be important factors in determining the best barrier site. The sites considered in this study are rather arbitrary, in that there was no consideration of other economic factors, such as construction costs. For

example, the Cape Sharpe site may be undesirable not only because the channel is very deep there, but the currents are also very large, so that closure operations for the barrier would be extremely difficult. There are secondary considerations also. For example, if the Cape Sharpe site is chosen, there is the possibility of building a causeway over the top of the barrier; this would save many tourists the long and tiring drive presently required to go around Minas Basin.

In conclusion, we have attempted only to estimate the decrease in tidal amplitude due to the presence of complete tidal barriers. In considering the feasibility of such a development, economical, political, engineering, and other considerations may override this consideration, but these are not within the realm of this study.

ACKNOWLEDGMENTS

The author is indebted to Mr F. G. Barber for his assistance in assembling the original manuscript. Dr G. Godin provided encouragement and advice while the work was being carried out. Mr M. Isabelle is commended for providing the line drawings both in this paper and in the original manuscript.

Thanks are extended to Mr G. C. Dohler, of the Tides and Water Levels Section, Marine Sciences Branch, Ottawa, for many fruitful discussions on the topic and for providing the analyzed tidal constituents.

The author is further indebted to Dr G. F. D. Duff, Chairman, Department of Mathematics, University of Toronto, for enlightening discussions on the subject, and to Mr F. Lawton of the Atlantic Tidal Power Programming Board.

REFERENCES

BRETTSCHNEIDER, G. 1963. Anwendung eines im Institut für Meereskunde der Universität Hamburg entwickelten Verfahrens zur numerischen Ermittlung von Gezeiten in Ramdmeeren auf die M_2 — Mitschwingungsgezeit der Nordsee. Als Diplomarbeit in Fach Ozeanographie des Mathematisch-Naturwissenshaftlichen Fakultät an der Universität Hamburg vorgelegt von Gottfried Brettschneider, Hamburg. 129 p.

DAWSON, W. B. 1917. Tides at the head of the Bay of Fundy. Dept. Naval Serv., Ottawa, Ont. 36 p.

GREENBERG, D. A. 1969. Modification of the M_2 tide due to barriers in the Bay of Fundy. J. Fish. Res. Bd. Canada. (Submitted for publication.)

HARRIS, D. L., AND C. P. JELESNIANSKI. 1964. Some problems involved in the numerical solutions of tidal hydraulics equations. Monthly Weather Rev. 92: 409–422.

INTERNATIONAL PASSAMAQUODDY ENGINEERING BOARD. 1961. Report of the International Joint Commission United States and Canada on the International Tidal Power Project, Washington–Ottawa. Rept. Intern. Joint Comm. Docket 72, Invest. Intern. Passamaquoddy Eng. Fish. Bd., April 1961.

INTERNATIONAL PASSAMAQUODDY FISHERIES BOARD. 1959. International Passamaquoddy Fisheries Board Report to the International Joint Commission. Appendix 1, Oceanography. Rept. Intern. Joint Comm. Docket 72, Invest. Intern. Passamaquoddy Eng. Fish. Bd., April 1961.

LANGFORD, C. J. MS, 1966. Bay of Fundy tidal report on tidal and current survey, 1965. Data Rept. BIO 66-2-D, Bedford Institute of Oceanography, Dartmouth, N.S.

MIYAZAKI, M. 1965. Numerical computation of the storm surge of Hurricane Carla, 1961, in the Gulf of Mexico. Oceanographical Mag. 17: 109–140.

RAO, D. B. 1968. Natural oscillations of the Bay of Fundy. J. Fish. Res. Bd. Canada 25: 1097–1114.

YUEN, K. B. MS, 1967. The effect of tidal barriers upon the M_2 tide in the Bay of Fundy. MS Rept. Ser. 5, Marine Sciences Branch, Dept. Energy, Mines Resources, Ottawa, Ont.

Effects of Fluctuations of Lead, Temperature, and Dissolved Oxygen on the Growth of Brook Trout[1]

D. DORFMAN[2] AND W. R. WHITWORTH

The University of Connecticut, College of Agriculture
Storrs, Conn. 06268, USA

DORFMAN, D., AND W. R. WHITWORTH. 1969. Effects of fluctuations of lead, temperature, and dissolved oxygen on the growth of brook trout. J. Fish. Res. Bd. Canada 26: 2493–2501.

Slug doses of lead administered once a day 5 days/week to brook trout exposed to simultaneous diel fluctuations of dissolved oxygen and temperatures showed that concentrations of 25 mg/liter of lead reduced growth. Concentrations of 15 mg/liter had little apparent effect and concentrations of 10 mg/liter had no deleterious effects on growth.

Received December 30, 1968

INTRODUCTION

THE PRIMARY PURPOSE of this study was to measure the effects of simultaneous variation of temperature and concentrations of lead and dissolved oxygen on the growth of brook trout, *Salvelinus fontinalis* (Mitchill). Few long-term studies of the chronic toxicity of lead have been made, i.e., subtle changes in appetite, metabolism, reproduction, or other vital functions of fishes exposed to lead for more than 96 hr. Dilling and Smith (1926) found that plaice exposed to 4 mg Pb/liter for 156 days had reduced growth. Dawson (1935) reported brown bullheads survived 16–183 days in water that contained 27.3 mg Pb/liter but became anemic. Ellis (1937) reported goldfish survived lead concentrations of 5.5 mg/liter for 61 days. Crandall and Goodnight (1963) exposed guppies to 1.24 and 3.12 mg Pb/liter for up to 129 days and reported histological changes, retarded gonadal development, and reduced growth. Chronic and acute toxicity concentrations obtained by continual exposure of fish to toxicants are often not as useful as those obtained from exposure of fish to slug doses of a toxicant because few toxicants are present in waters at constant concentrations 24 hr a day; industrial pollutants, such as metallic wastes, are often introduced into the water as slug doses. Industrial wastes may fluctuate widely (hourly and daily) in volume and strength (Klein, 1966).

Concentrations of 0.1–75 mg Pb/liter have been reported as acutely toxic (lethal within 96 hr) to fish (McKee and Wolf, 1963). The variation was wide because: (1) a variety of dilution waters were used ranging from distilled (Carpenter, 1925; Ellis, 1937) to chemical analysis unreported (Jones, 1938);

[1]Contribution 351 of the Storrs Agricultural Experiment Station.

[2]Present address: Department of Environmental Science, Rutgers University, New Brunswick, New Jersey, USA.

Printed in Canada (J1325)

(2) different species of fish were tested (Tarzwell and Henderson, 1957); (3) concentrations of calcium present in the waters varied (Carpenter, 1930); (4) hardness of the waters varied (Department of Scientific and Industrial Research, 1962); (5) different temperatures were employed (Carpenter, 1926); (6) concentrations of dissolved oxygen varied (Department of Scientific and Industrial Research, 1960); (7) fishes were acclimated to different levels of dissolved oxygen (Lloyd, 1960); (8) the ratio of biomass of test animals to the quantity of available poison varied between the tests (Skidmore, 1964); and (9) forms of lead that were used varied (McKee and Wolf, 1963).

Acute toxicity of lead is caused by the destruction of the respiratory epithelium (Lloyd, 1965; Haider, 1964). Haider (1964) describes this process as a cauterization that results when the salt of a heavy metal comes in contact with albumin substances secreted by epidermal cells. The albumin, the oxide of the metal, and the acid with which the metal combines form a deposit. The acid is not firmly held in the deposit and is washed away. Lesions formed by deposits are readily apparent in the sensitive respiratory epithelium of the gills in high concentrations of heavy metal salts (Haider, 1964). Lloyd (1965) suggests that a buildup of lead occurs when the rate of metal ions entering the gill epithelium is greater than the rate at which they are removed by the blood stream. Carpenter (1927, 1930) and Schweiger (cited in Haider, 1964) suggest that the death of fish in lead solutions is due to suffocation caused by inhibition of breathing because of coagulated mucus and layers of dead cells about the gill epithelium.

Chronic toxicity is similar and, in addition to cauterization, there is resorption of lead causing a gradual impairment in the function of the liver, kidney, and spleen (Haider, 1964), anemia (Dawson, 1935), and metabolic lesions in the intestine resulting in poor food utilization and reduced growth (Crandall and Goodnight, 1963).

MATERIALS AND METHODS

Brook trout (hatched January, 1967) were obtained from the State Fish Hatchery, Burlington, Connecticut, in March 1967. They were held in two 500-liter polyethylene tanks at 10 C and fed commercial trout pellets daily. Mortality was less than 1% for the entire period that they were held.

All waters, except those used in the preparation of stock solutions, were prepared by passing tap waters through deionizers and reconstituting them with salts (Lennon and Walker, 1964). Daily monitoring showed the specific conductances to be 257–303μ mho/cm at 25 C, alkalinities 27–34 mg/liter as $CaCO_3$, and pH's 6.8–7.2.

The fish were exposed to simultaneous variations of temperature and concentrations of dissolved oxygen and lead in the system described by Whitworth (1968). Waters, maintained at 10 C by an immersion cooler, were pumped from a 500-liter reservoir to the top of two marble-filled columns at a rate sufficient to keep them full, the excess being returned to the reservoir through overflows. Dissolved oxygen was reduced in one of the stripping columns by displacement with nitrogen gas, introduced at the base. The amount of oxygen removed was controlled by varying the rates of nitrogen and water flow. Diurnal fluctuations of dissolved oxygen were obtained by a device that alternately opened and closed nitrogen flow to the column. This provided low dissolved oxygen concentrations from 6 PM to 8 AM and air saturation concentra-

tions from 10 AM to 4 PM. Waters from the bases of the columns flowed to equilibrium chambers immersed in the water baths, then to 3.7-liter test chambers. One water bath was maintained at 10 C by an immersion cooler. A second water bath maintained a diurnal fluctuation of temperature controlled by an immersion cooler that operated from 7 PM to 5 AM. The temperature in the water bath was low from 7 PM to 5 AM and rose to a high by 7 PM when the cooler was turned on. Each test chamber was sealed with a rubber stopper fitted with two glass tubes for water entry and exit. Waters were delivered to each test chamber at rates rarely less than 20 or more than 44 ml/min. Flow rates were controlled by capillary tubes in the outflows of the equilibrium chambers. Water lines were constructed of tygon and glass tubing.

Dissolved oxygen content of the water, measured by the Alsterberg Modification of the Winkler Method (American Public Health Association et al., 1965), was determined daily (water samples obtained from outlets provided in the equilibrium chambers). The rubber stopper in each of several of the test chambers had a small opening through which a thermister lead was permanently placed. Temperature was measured several times each day in these chambers and in the water baths.

Six experiments were performed from April to October 1967. Experimental designs were randomized complete blocks with factorial arrangement of treatments (two or three concentrations of lead, two concentrations of dissolved oxygen, and one or two temperatures). Treatment combinations (Table 1) were replicated a minimum of three times for each test with one to three fish per replication.The system was operated for 1 or 2 days before the start of each experiment at arbitrarily selected treatment combinations. Solutions (made with ACS grade chemicals) of lead (10 g lead nitrate and 1 ml nitric acid diluted to 1 liter with deionized water), nitric acid (1 ml nitric acid diluted to 1 liter with deionized water), or deionized water (1 liter of deionized water) were added to the test chambers between 11 and 1 PM each day by pipetting 6–15 ml from the appropriate stock solutions into the bottom of each chamber (nitric acid concentrations were equal to the acid concentration in the highest lead treatment). This provided an instantaneous concentration in the test chambers, which was immediately diluted; the average flushing rate of the test chambers was 129 min (Table 1). Lead was not added weekends during the sixth experiment. Lead precipitates formed approximately 3 min after lead was added, i.e., the waters became cloudy and remained so for approximately 15 min. Trout appeared distressed while the cloudiness persisted. Precipitates adhered to the sides and base of each test chamber after about three treatments. These precipitates could be removed from the chambers only with nitric acid, and were heavy at 25 mg Pb/liter, light at 15 mg Pb/liter, and faint at 10 mg Pb/liter. Analysis of these precipitates (mass spectography and X-ray crystallography) indicated that the precipitates of lead adhering to the glass were probably lead sulfate. Solubility products, calculated on the molarity of the salts added to the deionized waters and considering the activity of all the ions present, indicated precipitates of lead occurred at all treatment combinations tested.

Fish, first graded for size and then individually selected at random within a group, were weighed at the beginning of each experiment (in a water-filled plastic container) then placed into the test chambers (one to three fish/chamber). Weights were recorded to the nearest 0.05 g. Specimens that died within 2 days after being introduced into the chambers were replaced, except that in the sixth experiment fish were replaced up to the 9th day of the 20-day experiment. Fish were fed and observed several times each day, and weighed at 3- to 7-day intervals. Chambers were cleaned at least once a week. Experiments were usually terminated when none of the fish remained in one of the treatment combinations. The adipose fin of one of the two fish in each chamber was clipped to determine the growth of each fish in the third experiment. Growth through the 9th day was compared by a group t-test (Ostle, 1964) and was not affected by finclipping ($P < 0.05$).

Laboratory temperature was kept at approximately 23.5 C with an air conditioner; room lighting was controlled by covering windows and using artificial lights from 6 AM to 6 PM.

Recorded weights of all fish, except those that died in any treatment combination before the 2nd day, were converted to percent initial body weight. A linear relationship (log–log) was assumed between percent initial body weight and time; preliminary charting of all treatments

TABLE 1. Summary of experimental conditions and results.

Exptl. conditions	Treatment combination	Oxygen (*mg/liter*)	Temp (*C*)	Pb (*mg/liter*)	Flush rate (*ml/min*)	Initial wt of fish (*g*)	Regression coefficients[a] (b_0+b_1)
Experiment 1							
3 fish/chamber, 20 chambers							
18 test days, 3 feedings/day	1	C[b]	C[b]	0	156(85–269)	2.00(1.10–2.95)	61.7+1.7*
Oxygen C = 10.5(9.9–11.1)	2	F[c]	C	0	135(85–159)	1.70(1.30–2.30)	86.2+1.0**
(*mg/liter*) F = 2.4(2.0–2.9)	3	C	C	25	153(103–268)	2.15(1.55–3.30)	92.3+0.9**
to 10.5(9.9–11.1)	4	F	C	25	129(69–209)	1.75(1.20–2.90)	117.4+0.8**
Temp (*C*) C = 10.5(9.9–11.1)							
Experiment 2							
3 fish/chamber, 20 chambers							
48 test days, 6 feedings/day	1	C	C	0	104(81–155)	1.95(1.45–3.00)	41.0+3.1*
Oxygen C = 10.6(9.7–11.3)	2	F	C	0	110(82–143)	2.05(1.40–2.60)	47.9+2.0**
(*mg/liter*) F = 3.2(2.4–4.9)	3	C	C	25	103(78–145)	2.10(1.55–2.55)	70.4+1.4**
to 10.6(9.7–11.3)	4	F	C	25	105(78–154)	2.00(1.30–2.90)	103.4+0.9***
Temp (*C*) C = 10.0(8.5–10.2)							
Experiment 3							
	1	C	C	.01HNO_3[d]	113(93–156)	5.20(4.00–7.00)	50.2+2.4*
2 fish/chamber, 36 chambers	2	C	C	10	122(93–156)	5.20(4.15–7.30)	48.1+2.0*
38 test days, 7 feedings/day	3	C	F[e]	10	138(105–157)	5.05(3.95–8.10)	60.4+1.9*
Oxygen C = 10.5(9.5–11.5)	4	F	C	10	105(98–125)	5.40(3.50–7.20)	63.6+1.8*
(*mg/liter*) F = 6.5(4.8–7.8)	5	F	F	.01HNO_3	124(93–157)	6.30(4.45–7.55)	66.2+1.6*
to 10.5(9.5–11.5)	6	F	F	10	120(93–154)	5.00(4.00–7.10)	73.7+1.5*
Temp (*C*) C = 9.5(8.5–10.2)	7	C	F	.01HNO_3	135(93–156)	5.10(4.30–6.65)	89.5+1.2**
F = 9.5(8.5–10.2)	8	F	C	.01HNO_3	125(103–129)	6.75(4.05–8.85)	87.6+1.1**
to 15.2(14.8–15.5)	9	C	F	25	137(111–157)	5.25(3.35–6.70)	83.9+1.1**
	10	F	C	25	113(93–143)	5.25(4.35–6.50)	91.9+1.1**
	11	C	C	25	117(93–155)	5.35(3.95–7.20)	86.9+1.0**
	12	F	F	25	131(109–157)	4.45(3.70–5.35)	100.0+0.9**
Experiment 4							
	1	C	C	15	132	7.40(6.95–8.25)	81.2+1.5
1 fish/chamber, 36 chambers	2	F	F	0	154	7.85(7.75–8.05)	79.4+1.4
10 test days, 5 feedings/day	3	F	F	.006HNO_3	155	7.50(6.85–8.55)	85.9+1.4

1 fish/chamber, 36 chambers		1	F	F	.006HNO$_3$	131(109–168)	16.60(15.20–17.95)	68.9+1.5**
15 test days, 3 feedings/day		2	C	C	15	138(110–186)	17.75(16.15–19.75)	68.6+1.5**
Oxygen	C = 10.7(10.3–11.2)	3	C	C	0	135(109–155)	17.40(15.25–20.05)	79.1+1.4**
(*mg/liter*)	F = 6.5(6.0–7.2)	4	F	C	0	161(98–155)	16.60(14.95–17.65)	85.5+1.4**
	to 10.7(10.3–11.2)	5	C	F	0	135(104–170)	17.35(16.90–17.95)	78.4+1.3**
Temp (*C*)	C = 9.8(9.5–10.0)	6	F	C	15	116(98–157)	15.60(15.30–16.05)	84.2+1.3**
	F = 9.8(9.5–10.0)	7	F	F	0	131(109–169)	17.60(17.10–18.50)	74.2+1.3**
	to 15.2(14.8–15.5)	8	F	F	15	142(118–171)	17.20(15.40–19.00)	80.6+1.2**
		9	F	C	.006HNO$_3$	135(104–170)	14.40(13.50–15.50)	96.1+1.2**
		10	C	C	.006HNO$_3$	142(117–187)	17.15(16.60–17.65)	88.8+1.2**
		11	C	F	15	160(144–187)	15.95(14.50–16.90)	88.9+1.1**
		12	C	F	.006HNO$_3$	148(111–171)	16.20(15.75–16.65)	100.7+1.0**
Experiment 6								
		1	F	F	15	116(92–185)	22.95(15.95–34.25)	99.4+1.1
1 fish/chamber, 24 chambers		2	F	C	15	107(77–205)	27.70(20.00–41.40)	88.6+1.1
20 test days, 2 feedings/day		3	C	F	15	98(77–205)	24.95(20.45–29.25)	91.2+1.0
Oxygen	C = 10.5(9.8–10.8)	4	C	F	0	102(77–154)	27.80(22.65–34.00)	95.0+1.0
(*mg/liter*)	F = 4.5(3.8– 4.8)	5	C	C	0	109(84–154)	26.30(11.40–39.00)	94.5+1.0
	to 10.5(9.8–10.8)	6	F	C	0	104(77–205)	26.20(20.65–32.25)	95.3+1.0
Temp (*C*)	C = 9.8(8.5–10.0)	7	C	C	15	102(77–154)	28.20(23.50–30.65)	104.2+0.9
	F = 9.8(8.5–10.0)	8	F	F	0	129(92–231)	24.85(15.45–32.40)	105.8+0.8
	to 15.2(14.8–15.5)							

[a]All treatment combinations that are not significantly different ($P < 0.05$) in slope (covariance analysis) are marked with the same number of asterisks (*).

[b]C = constant oxygen concentration or water temperature.

[c]F = fluctuating oxygen concentration or water temperature.

[d]HNO_3 is reported in ml/liter.

on logarithmic paper confirmed this. Treatment combinations within each experiment were analyzed by covariance analysis (Snedecor and Cochran, 1967). Treatment combinations were analyzed for only those dates on which at least two fish were included. A second regression line was calculated separately for trout exposed to 25 mg Pb/liter, 10.5 mg/liter dissolved oxygen, and 9.5 C from the 30th to the 38th days in the third experiment. Treatment combinations in the fourth and sixth experiments were not compared by covariance analysis because there were not enough observations. The fourth experiment was terminated after 10 days because of a malfunction in the apparatus. Slime, caused by bacterial growth around the rubber stoppers in several of the equilibrium chambers, loosened the stoppers and halted the flow of waters to the test chambers. This resulted in the death of trout in many chambers. Some of the large trout in some treatment combinations in the sixth experiment died and had to be replaced, causing a loss in the number of observations. A program was written for the IBM 360 to perform the covariance analysis and obtain the adjusted means (arranged in descending order in tabular form), the differences between the adjusted means, and the standard deviations necessary to perform a sequential test (Snedecor and Cochran, 1967).

Ninety-six hour TL_m's (acute toxicity) were obtained by graphical interpolation (American Public Health Association et al., 1965) for each of the six experiments previously described and from one additional experiment in which two trout (8.0–9.9 g) were exposed to each of six concentrations of lead (0, 1.56, 3.12, 6.25, 12.60, and 18.75 mg Pb/liter) at 9.6 C, in 18.5-liter glass chambers filled with 6 liters of a lead solution, for 4 days. Each lead level was duplicated twice in the latter test; the 0 lead level contained 0.03 ml/liter HNO_3.

RESULTS

All treatment combinations that included a lead concentration of 25 mg/liter administered once/day as a slug dose reduced growth of brook trout. Thirty-two of the 84 trout treated died within 15 days. Some trout (14 specimens) in all treatment combinations including 25 mg Pb/liter, except those including temperatures that fluctuated between 9.5 and 15.7 C, had growth rates comparable to those of fish in other treatment combinations after 30 days, i.e., in the third experiment the slope of the line for trout exposed to 25 mg Pb/liter, 10.5 mg/liter dissolved oxygen at 9.5 C, was 2.0 compared with 2.4 for the "controls". Treatment combinations that included 15 mg Pb/liter administered once a day (5 days/week) as a slug dose had no apparent effect on the growth of the trout. Trout were not adversely affected in any treatment combinations including 10 mg Pb/liter administered as a slug dose and generally had better growth than those in the treatment combinations including HNO_3.

Trout exposed to treatment combinations including no lead that included dissolved oxygen concentrations of both 2.4–10.6 and 3.5–10.6 mg/liter did not grow as well as those in treatment combinations including dissolved oxygen concentrations of 10.6 mg/liter in the first and second experiments respectively. Treatment combinations including fluctuating dissolved oxygen concentrations of approximately 6.5–10.7 mg/liter probably did not affect growth. Trout grew better in treatment combinations including 9.5 C than in those fluctuating between 9.5 and 16.0 C.

Interactions of oxygen × lead were indicated by the covariance analysis in experiments 1–3 but not in experiments 4 and 5. Interactions of temperature × lead and oxygen × temperature were indicated in experiments 3, 4, and

5. An interaction of oxygen × temperature × lead was suggested by experiment 3 (the least growth obtained was in the treatment combination receiving 6.5–10.5 mg/liter of dissolved oxygen, 9.5–15.7 C, and 25 mg Pb/liter) but not in experiments 4 and 5. Generally, better growth was achieved when temperature was constant and dissolved oxygen was constant.

The trout exposed to constant levels of lead for 4 days had a 96-hr TL_m of 3.12 mg Pb/liter, whereas the 96-hr TL_m's for trout exposed to all treatment combinations including a slug dose of lead, were greater than the highest concentrations tested (15 or 25 mg Pb/liter). All trout exposed to 0.03 ml/liter of nitric acid in the acute bioassay survived.

DISCUSSION

Although it was our intent to expose fish to a slug-dose type of effluent, we were unable to do so because of the precipitates that formed in all treatment combinations including lead, e.g., the fish may have been constantly exposed to low levels of lead that redissolved from the walls of the test chamber. This may be similar to what might occur in a "natural" situation. The only areas of streams that would probably receive slug doses of lead that would reduce growth of trout (25 mg/liter) would be encountered in effluent sources and for a limited distance downstream. This distance would depend upon the concentration of sulfates and water temperatures since an increase in sulfate concentration and a decrease in temperature each decrease the amount of lead in solution. This zone would be extended if insoluble lead were carried downstream and redissolved. For example, trout, or other fishes, in streams that received lead discharges that are usually precipitated because of low temperatures and low concentrations of sulfate might be exposed to slug doses of lead well above 25 mg/liter as the precipitated lead redissolves on a hot summer day after a rainstorm. This indirect effect of lead, possibly many miles from the source of the pollution, should be considered when allowable concentrations of lead pollution are established. Although Carpenter (1927) and Wallen et al. (1957) reported only ionic lead as acutely toxic to fish, Ellis (1937) reported finely powdered lead sulfide (insoluble in water) applied to the bottom of glass test chambers killed fish after 61 days of exposure. Theoretically rivers in New England could contain more than 25 mg Pb/liter in solution because sulfate concentrations of 11–16 and 7.3–11.5 mg/liter and temperatures of 28 and 31 C have been reported in the waters of the Connecticut and Housatonic river basins, respectively (Anon., 1967). Summer temperatures in these rivers are too high to support large populations of brook trout; therefore, the amount of lead in solution in these two areas would be of secondary importance to this species.

The size of the fish relative to the size of the test chamber is an important factor to consider (1) in future studies utilizing growth as a response, and (2) when extrapolating results of laboratory studies to "natural" waters. This interaction was probably responsible for masking the effects of the treatment combinations in the sixth experiment (the mean weight of the trout, 27.0 g,

was 10 g more than those in the fifth experiment and approximately 25 g more than those in the first experiment), whereas the growth rate of the 1- to 8-g brook trout (considering the effects of treatment combinations containing diel fluctuations of dissolved oxygen from both 2 4 and 3.5 to approximately 10.5 mg/liter) was similar to that reported for 1.6-g coho salmon (Herrmann et al., 1962; Fisher, MS, 1963). The low growth rate of these large trout exhibited in the sixth experiment in similar treatment combinations was similar to that reported by Whitworth (1968) who exposed 20- to 34-g brook trout to diel fluctuations of dissolved oxygen. Using more than one small fish per test chamber should be discouraged in future studies because more than one 5-g fish per test chamber (experiment 3) resulted in fish establishing territories and attacking each other; 2-g fish (experiments 1 and 2) did not exhibit this response.

ACKNOWLEDGMENTS

This study was supported, in part, by funds provided by the U.S. Department of the Interior under the Water Resources Research Act of 1964, Public Law 88-379. Dr J. J. Lucas, University of Connecticut, advised regarding the statistical analysis of data; Mr Maynard Hunt, Air Force Cambridge Research Laboratory, Bedford, Massachusetts, performed the mass spectographic analysis; and Dr L. Katz, University of Connecticut, the X-ray crystallography. Fish were provided by the Connecticut State Board of Fisheries & Game. Work performed by the Computer Center was supported, in part, by funds provided by the National Science Foundation Grant 6P-1819.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1965. Standard methods for the examination of water and waste water including bottom sediments and sludges. 12th ed. Public Health Assoc., Inc. New York. 769 p.

Anon. 1967. Water resources data for Connecticut. U.S. Dept. Interior, Geol. Surv. Water Res. Div. 172 p.

Carpenter, K. E. 1925. On the biological factors involved in the destruction of river fisheries by pollution due to lead-mining. Ann. Appl. Biol. 12: 1–13.
1926. The lead mine as an active agent in river pollution. Ann. Appl. Biol. 13: 395–401.
1927. The lethal action of soluble metallic salts on fishes. Brit. J. Exptl. Biol. 4: 378–390.
1930. Further researches on the action of metallic salts on fishes. J. Exptl. Zool. 56: 407–422.

Crandall, C. A., and C. J. Goodnight. 1963. The effects of sublethal concentrations of several toxicants to the common guppy, *Lebistes reticulatus*. Trans. Am. Microscop. Soc. 82: 59–73.

Dawson, A. B. 1935. The hemopoietic response in the catfish, *Ameirus nebulosus*, to chronic lead poisoning. Biol. Bull. 68: 335–346.

Department of Scientific and Industrial Research. 1960. Water pollution research, 1959. Her Majesty's Stationery Office, London. 115 p.
1962. Water pollution research, 1961. Her Majesty's Stationery Office, London. 127 p.

Dilling, W. J., and W. C. Smith. 1926. Experiments on the effects of lead on the growth of plaice (*Pleuronectes platessa*). Ann. Appl. Biol. 13: 168–176.

Ellis, M. M. 1937. Detection and measurement of stream pollution. Bull. U.S. Bur. Fish. 48: 365–437.

Fisher, R. M. MS, 1963. Influence of oxygen concentration and its diurnal fluctuations on the growth of the juvenile coho salmon. M.Sc. Thesis. Oregon State University, Corvallis, Ore. 48 p.

HAIDER, G. 1964. [Heavy metal poisoning in fish. 1. Demonstration of lead poisoning in rainbow trout (*Salmo gairdneri* Rich.)] (Transl. from German.) Z. Angew. Zool. 51: 347–368.

HERRMANN, R. B., C. E. WARREN, AND P. DOUDOROFF. 1962. Influence of oxygen concentration on the growth of juvenile coho salmon. Trans. Am. Fish. Soc. 91: 155–167.

JONES, J. R. E. 1938. The relative toxicity of salts of lead, zinc, and copper to the stickleback (*Gasterosteus aculeatus* L.) and the effect of calcium on the toxicity of lead and zinc salts. J. Exptl. Biol. 15: 394–407.

KLEIN, L. 1966. River pollution 3: Control. Butterworths, London. 484 p.

LENNON, R. E., AND C. R. WALKER. 1964. Investigations in fish control: 1. Laboratories and methods for screening fish-control chemicals. Bur. Sport Fish. Wildlife Circ. 185. 15 p.

LLOYD, R. 1960. The toxicity of zinc sulphate to rainbow trout. Ann. Appl. Biol. 48: 84–94.
1965. Factors that affect the tolerance of fish to heavy metal poisoning, p. 181–187. *In* Biological problems in water pollution: Transactions of the 1962 seminar. Public Health Serv. Publ. 999-WP-25. Robert A. Taft Sanitary Engineering Center, U.S. Public Health Serv., Cincinnati, Ohio.

MCKEE, J. E., AND W. W. WOLF. 1963. Water quality criteria. Calif. Water Quality Control Bd. Publ. 3-A. 548 p.

OSTLE, B. 1964. Statistics in research. 2nd ed. Iowa State Univ. Press, Ames, Iowa. 585 p.

SKIDMORE, J. F. 1964. Toxicity of zinc compounds to aquatic animals with special reference to fish. Quart. Rev. Biol. 39: 227–248.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods. Iowa State Univ. Press, Ames, Iowa. 593 p.

TARZWELL, C. M., AND C. HENDERSON. 1957. Bioassays for control of industrial effluents. Sewage Ind. Wastes 29: 1001–1017.

WALLEN, I. E., W. C. GREER, AND R. LASATER. 1957. Toxicity of *Gambusia affinis* of certain pure chemicals in turbid waters. Sewage Ind. Wastes 29: 695–711.

WHITWORTH, W. R. 1968. Effects of diurnal fluctuations of dissolved oxygen on the growth of brook trout. J. Fish. Res. Bd. Canada 24: 579–584.

Influence of Temperature on Gaffkemia, a Bacterial Disease of the Lobster *Homarus americanus*

JAMES E. STEWART, JOHN W. CORNICK, AND B. M. ZWICKER

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

STEWART, JAMES E., JOHN W. CORNICK, AND B. M. ZWICKER. 1969. Influence of temperature on gaffkemia, a bacterial disease of the lobster *Homarus americanus*. J. Fish. Res. Bd. Canada 26: 2503–2510.

The mean time to death for lobsters (*Homarus americanus*) infected with *Gaffkya homari* and kept at constant temperatures was 2 days at 20 C, 12 days at 15 C, 28 days at 10 C, 65 days at 7 C, 84 days at 5 C, and 172 days at 3 C. The lobsters were unable to mobilize at any of these temperatures systemic defenses adequate to eliminate the pathogen. No deaths that could be attributed to infection with *G. homari* occurred during the 250-day trial at 1 C; the pathogen, however, was not eliminated at this temperature but remained in the lobsters at low levels with virulence unchanged and gave rise to a fatal infection when the temperature was increased. Further studies showed that infected lobsters were adversely affected by a relatively rapid increase of 10 degrees C but not by a 5-degree C increase; a relatively rapid decrease of 15 degrees C but not 10 degrees C was adverse. Infected lobsters at 15 C were most sensitive to a 5-degree C decrease around the 12th day of the infection.

Received April 29, 1969

INTRODUCTION

TEMPERATURE is a key factor in all aspects of a poikilotherm's existence and becomes even more important in any interaction between a poikilotherm and its parasite. Feng and Stauber (1968) emphasize this in their statement regarding such interactions, "the thermal effect may retard the growth of microorganisms, influence the production of metabolites and alter the nutritional requirement. To a host, the cellular and humoral mechanisms of defense may be enhanced at the optimum temperature for that given host species." Thus the outcome of a parasitic incident could be decided by the ambient temperature favoring one of the protagonists.

Thus, the influence of temperature on host–parasite interactions is basic to a complete understanding of the relationship. Accordingly in this study on gaffkemia, the septicemic disease of the lobster *Homarus americanus*, caused by the bacterium *Gaffkya homari* (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947; and Roskam, 1957) we have determined the effect of temperature.

METHODS

LOBSTERS

Both large chelae of each lobster (*Homarus americanus*) were closed with rubber bands immediately after the lobsters were captured from the Nova Scotian littoral. All lobsters (546 ±

Printed in Canada (J1462)

23 g) were acclimated to the desired temperature for a minimum of 2 weeks and were examined and found to be free of *G. homari* before being included in an experiment. None of the lobsters was fed during the acclimation or the experimental periods. All live animal experiments, except those at 1 and 3 C, were carried out in tanks supplied with aerated, filtered and recirculated sea water at a constant temperature (within 0.5 degree C of the desired temperature). Each Monday, Wednesday, and Friday during the course of the experiments 80% of the water in these tanks was replaced with new sea water, previously heated or cooled to the tank temperature. The experiments at 1 and 3 C were carried out in refrigerated tanks specially constructed for these experiments and equipped with elaborate controls; these tanks were supplied with a constant flow of new, filtered sea water and kept within 0.5 degree C of the specified temperature. Individual shelters, which the lobsters could use at will, were provided in all tanks. Care was taken to ensure that the tanks of control animals were not subject to infection from the tanks of infected animals.

Bacteriological

The strain of *G. homari* used in these experiments was isolated originally by Dr Rabin from a diseased lobster. Virulence was ensured by continuous passage of this strain through lobsters held at 15 C. Methods used for the isolation and identification of *G. homari* have been described previously (Stewart et al., 1966). Trypticase Soy (TS) broth or agar was used for all in vitro microbiological work at an incubation temperature of 28 C.

The infective cell suspensions were prepared under aseptic conditions. Trypticase Soy broth (50 ml) was inoculated with 0.2 ml of hemolymph drawn from a lobster infected with *G. homari* (a continuous passage animal) and then incubated for 24 hr at 28 C. The bacterial cells were harvested, washed three times with sterile 3% NaCl, and suspended for immediate use in sterile 3% NaCl at an optical density of 0.1 (ca. 5×10^6 bacteria/ml) determined at 420 mμ using a Bausch and Lomb Spectronic 20 spectrophotometer (12-mm cuvettes). The suspensions were checked for purity. Viable numbers in the suspensions and other bacterial counts were determined by the plate-count method of Miles and Misra (1938). The experiment proper began with the injection into the ventral abdominal sinus of each acclimated lobster of a standard volume (1.0 ml/kg live body wt) of sterile 3% NaCl for the control lobsters and the standardized *G. homari* suspension for the "infected" group. The site was swabbed thoroughly with 70% ethanol before and after injection.

RESULTS

The mean time to death (MTD) for lobsters infected with *G. homari* is directly related to temperature (Table 1); the lower the temperature the longer the time to death. The MTD for the 15-C trial reported in Table 1 is shorter than the average of 17 days reported for 12 separate trials at this temperature by Cornick and Stewart (1968); they reported that the MTD, however, in the individual trials ranged from 9 to 21 days. Variation between lobsters from different groups and areas could account for a wide variation in MTD. Thus the MTD should not be considered in absolute terms but in relative terms that also give an indication of magnitude. During the 250-day trial at 1 C the number of deaths among the control group was approximately equal to the number for the infected group; this, plus the fact that the numbers of *G. homari* found at death in 11 of the lobsters originally injected with it were less than 10/ml of hemolymph did not permit us to attribute any of the deaths at 1 C to gaffkemia.

Hemolymph samples were drawn periodically from the lobsters held at 1 and 3 C during the 250-day trial. *Gaffkya homari* could be detected only in

TABLE 1. MTD for lobsters infected with *Gaffkya homari* and held at constant temperatures.

Temp (*C*)	Treatment[a]	No. lobsters	No. dead	No. dead infected	No. survivors	No. survivors patently infected	MTD±SE (*days*)	*G. homari*: no. at death per ml	Observation period (*days*)
1	K	52	14	0	38	0	154[b]		250
	I	52	15	11	37	27	202[b]	<10	250
3	K	52	12	0	40	0	210[b]		250
	I	52	43	41	9	9	172±6.4	2.9×10^7	250
5	K	10	0	0	10	0	–		133
	I	10	9	9	1	1	84±6.7	1.7×10^7	133
7	K	10	0	0	10	0	–		86
	I	10	9	9	1	1	65±6.4		86
10	K	10	0	0	10	0	–		48
	I	10	10	10	0	0	28±2.4		48
15	K	10	0	0	0	0	–		21
	I	10	10	10	0	0	12±1.6	ca. 10^9[c]	21
20	K	10	0	0	10	0	–		5
	I	10	9	9	1	1	2±0.3		5

[a]K = control; I = infected.
[b]None of these MTD values were attributed to gaffkemia.
[c]From Stewart et al. (1969a).

approximately half of the 1-C lobsters injected with it and only then in small numbers (less than 10/ml of hemolymph); lobsters from which *G. homari* were isolated at one sampling might be completely negative at the next sampling and vice versa. In contrast the lobsters injected with *G. homari* and held at 3 C were all shown to have the pathogen in their hemolymph from the early stages of the experiment; after an initial "fading" of the pathogen the numbers increased logarithmically (Fig. 1).

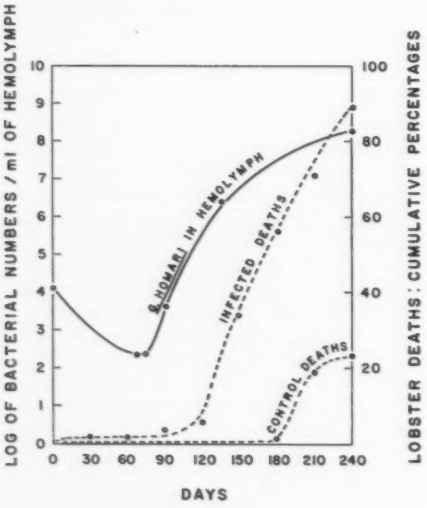
LOG OF BACTERIAL NUMBERS / ml OF HEMOLYMPH
LOBSTER DEATHS: CUMULATIVE PERCENTAGES
DAYS
G. HOMARI IN HEMOLYMPH
INFECTED DEATHS
CONTROL DEATHS
0 30 60 90 120 150 180 210 240
0 1 2 3 4 5 6 7 8 9 10
0 20 40 60 80 100

FIG. 1. Growth of *G. homari* in the hemolymph of lobsters injected with the pathogen and held at 3 C, plus the cumulative percentages of deaths in the control and infected groups.

At the end of the 250-day trial at 1 and 3 C the temperature of the water for half of the lobsters in the control groups and all of the survivors in the groups originally injected with *G. homari* was raised gradually to 15 C (over a 5-day period for the 1-C groups and a 3-day period for the 3-C groups). All of the lobsters in the control groups survived whereas all of the lobsters in the originally infected groups died with average *G. homari* counts of 3.5×10^8 organisms/ml of hemolymph. Checks at 4 and 8 days after temperature increase showed that all of the *G. homari*-injected lobsters were heavily infected. The MTD for both 1- and 3-C infected lobsters, dated from the beginning of the temperature increase, was 15 days. During and after the temperature increase those lobsters that at 1 C appeared to be uninfected, although originally injected with *G. homari*, were kept well separated from the others to prevent cross infection, which would occur only through wounds (Stewart et al., 1969b).

The influence of temperature on the MTD for lobsters infected with *G. homari* is consistent with the in vitro growth of the pathogen at the different temperatures (Fig. 2 and 3). The organism was able to grow at all of the trial temperatures and only at 1 C did the growth curve assume a radically different

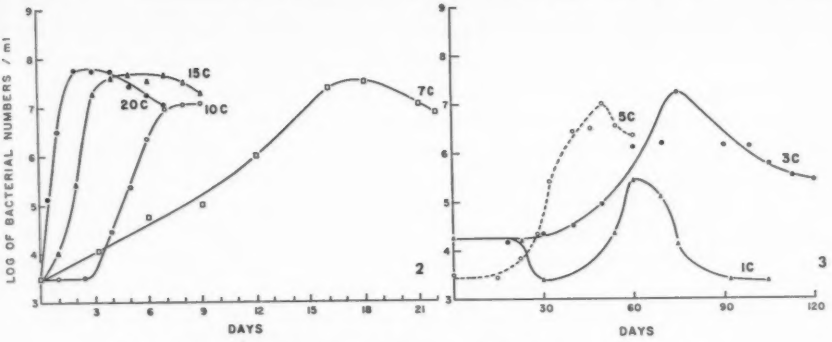

FIG. 2, 3. In vitro growth of *G. homari* (Trypticase Soy Broth).

shape. At 1 C the bacterial numbers declined around the 30th day followed by a logarithmic increase and a subsequent decline.

The results reported in Table 1 refer only to trials conducted at a constant temperature with lobsters acclimated to those temperatures. The natural environment, however, is not constant in temperature (Fig. 4); consequently, a complete assessment of the effect of temperature on the MTD for infected lobsters should include data on the effect of varying temperatures. The results of trials with varying temperatures are given in Table 2.

DISCUSSION

The most obvious effect of temperature on gaffkemia is that the MTD is greatly extended at the lower temperatures. Additionally the lobsters were unable at any of the temperatures to mobilize systemic defenses adequate to

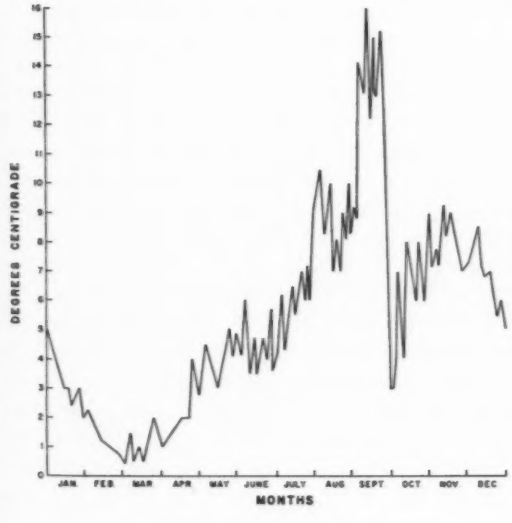

FIG. 4. Seawater temperatures for 1966 in the immediate vicinity of the Halifax laboratory (approximately 20 m depth).

TABLE 2. Effect of variations in temperature[a] on lobsters infected with *G. homari*.

Initial temp (*C*)	Treatment[b]	Period at initial temp after injection (*days*)	Final temp (*C*)	No. lobsters	No. dying[c]			MTD for period after temp change ±SE	Observation period (*days*)
					Before temp change	1 day after temp change	Final		
5	K	60	5	13	–	–	0	–	60
	I	60~	5	12	–	–	7	49±2.5[d]	60
	I	18	10	13	0	0	13	21±1.6	60
	I	18	15	13	0	0	13	4±0.24	60
	I	<1	15	13	0	0	13	11±1.1	60
15	K	6	10	5	0	0	0	–	22
	I	6	10	8	0	1	1	1	22
	K	9	10	5	0	0	0	–	22
	I	9	10	7	0	0	2	8	22
	K	12	10	10	0	0	0	–	22
	I	12	10	8	0	3	7	4±1.0	22
	K	15	10	5	1	1	1	–	22
	I	15	10	6	0	2	4	1±0.5	22
	K	10	5	10	0	0	0	–	26
	I	10	5	10	3	0	4	12	26
	K	10	0	6	0	0	0	–	32
	I	10	0	9	0	8	9	3±3.3	32

[a]Rate of temperature change equaled 1 degree C/hour.
[b]K = control; I = infected.
[c]None of the controls was infected at death; all of the others were.
[d]Total MTD since temperature was not changed.

eliminate the pathogen. Only at 1 C was the infection subpatent in contrast to the results reported by Feng and Stauber (1968) for hexamitiasis in oysters.

Although the numbers of *G. homari* apparent at any one time in the hemolymph in any of the 1-C "infected" lobsters were very low, the pathogen had been retained by all of them with unchanged virulence. This was revealed by the immediate and fatal infection that occurred in all of the 1-C "infected" lobsters when the temperature was increased at the end of the 250-day trial (very small numbers of *G. homari* are required to initiate an active infection, less than 5 bacteria/lobster (Cornick and Stewart, 1968)). The retention of *G. homari* by lobsters for 250 days at 1 C illustrates one mechanism whereby the pathogen could be carried from one warm-water season to the next and cause an epizootic the following year.

The numbers of control lobsters at 1 and 3 C (Table 1) that died were larger than we had expected. If the lobster has a cyclical requirement for variations in temperature, then a period at a constant and also very low temperature far greater than anything normally experienced, such as the reported 250-day period, might violate this requirement sufficiently to cause death.

The "fading" of the pathogen in the initial stages of the in vivo growth at 3 C (Fig. 1) has been observed also at 15 C (Stewart et al., 1969a). At 3 C as at 15 C the probable explanation is that the pathogen is phagocytosed with this event followed by bacterial growth in the phagocyte and the eventual destruction of the hemocyte releasing *G. homari* in large numbers into the hemolymph (Stewart et al., 1969a). The initial "fading" of the pathogen in vivo probably also occurs at all temperatures.

The in vitro growth at different temperatures of *G. homari* in TS broth (Fig. 2 and 3) is consistent with the MTD for infected lobsters. The fading noted in the in vivo growth does not occur in vitro except at 1 C. The growth is slower and less abundant in the TS broth at the lower temperatures; the less abundant growth was noted also in vivo at the lower temperatures where the terminal *G. homari* counts at 5 and 3 C were 90–99% lower than those at 15 C (Table 1). Another interesting point was the ability of *G. homari* to survive 100 days at 1 C in TS broth; this does not explain why the periodic epizootics occur but it does illustrate this organism's ability to survive extreme conditions. Our temperature records (1963–69) for the local sea water (approximately 20 m depth) are representative of much of the Canadian Atlantic lobster grounds. These indicate that since 1962 the sea water temperature has been 1 C or between 0 and 1 C for an average of 50 days annually (range 30–90 days). Thus the data indicate that *G. homari* can survive easily the range of temperatures experienced and their duration in this area.

The effect of changing the temperature after the lobsters had been infected at their temperature of acclimation (Table 2) indicates that a rapid increase of 10 C shortens the MTD materially. A 15-degree C decrease is immediately fatal to lobsters infected for an appreciable period and the most sensitive period to temperature change at 15 C occurs from around the 12th day of the infection

onward. The numbers of *G. homari* in the hemolymph at death in those experiments where this was determined were again consistently lower at the lower temperatures.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Mr B. Arie and Mr G. W. Horner. We also thank Dr Harvey Rabin, Institute for Comparative Biology, San Diego, California, for the strain of *G. homari*; Mr D. G. Ellis of our laboratory for the construction of the special refrigerated tanks that made the work at 1 and 3 C possible; Dr R. M. MacKelvie for his constructive criticism of the manuscript; and the Industrial Development Service of the Department of Fisheries of Canada for financial support for part of this work.

REFERENCES

CORNICK, J. W., AND J. E. STEWART. 1968. Interaction of the pathogen *Gaffkya homari* with natural defence mechanisms of *Homarus americanus*. J. Fish. Res. Bd. Canada 25: 695–709.

FENG, S. Y., AND L. A. STAUBER. 1968. Experimental hexamitiasis in the oyster, *Crassostrea virginica*. J. Invertebrate Pathol. 10: 94–110.

HITCHNER, E. R., AND S. F. SNIESZKO. 1947. A study of a microorganism causing a bacterial disease of lobsters. J. Bacteriol. 54: 48.

MILES, A. A., AND S. S. MISRA. 1938. The estimation of the bactericidal power of the blood. J. Hyg. 38: 732–749.

ROSKAN, R. TH. 1957. Gaffkaemia, a contagious disease in *Homarus vulgaris*. Conseil Perm. Intern. Exploration Mer, Shellfish Comm., Charlottenlund Slot, Danemark. 4 p.

SNIESZKO, S. F., AND C. C. TAYLOR. 1947. A bacterial disease of the lobster (*Homarus americanus*). Science 105: 500.

STEWART, J. E., B. ARIE, B. M. ZWICKER, AND J. R. DINGLE. 1969a. Gaffkemia, a bacterial disease of the lobster, *Homarus americanus*: Effects of the pathogen, *Gaffkya homari*, on the physiology of the host. Can. J. Microbiol. 15: 925–932.

STEWART, J. E., J. W. CORNICK, D. I. SPEARS, AND D. W. MCLEESE. 1966. Incidence of *Gaffkya homari* in natural lobster (*Homarus americanus*) populations of the Atlantic region of Canada. J. Fish. Res. Bd. Canada 23: 1325–1330.

STEWART, J. E., A. DOCKRILL, AND J. W. CORNICK. 1969b. Effectiveness of the integument and gastric fluid as barriers against transmission of *Gaffkya homari* to the lobster *Homarus americanus*. J. Fish. Res. Bd. Canada 26: 1–14.

Infectious Pancreatic Necrosis: Clinical and Immune Response of Adult Trouts to Inoculation with Live Virus

KEN WOLF AND M. C. QUIMBY

Bureau of Sport Fisheries and Wildlife
Eastern Fish Disease Laboratory
Kearneysville, West Virginia 25430, USA

WOLF, KEN, AND M. C. QUIMBY. 1969. Infectious pancreatic necrosis: clinical and immune response of adult trouts to inoculation with live virus. J. Fish. Res. Bd. Canada 26: 2511–2516.

Mature feral rainbow trout were inoculated with infectious pancreatic necrosis (IPN) virus and held at 12.5 C for 3 months to produce antibody and to determine whether or not disease would occur. Disease was not observed, but IPN virus-neutralizing antibody was found in pre-inoculation serum indicating the likelihood of prior infection. As measured by Neutralization Index, the immune response varied from weak to very strong. Neutralizing antibody was not found in sera from mature specific pathogen-free rainbow and brook trout. Although IPN virus also failed to produce clinical disease in adult brook trout it did establish a transient asymptomatic infection, and this resulted in high levels of neutralizing antibody.

Received April 17, 1969

INTRODUCTION

INFECTIOUS PANCREATIC NECROSIS (IPN) is the most prevalent of the known viral diseases of North American salmonids. It occurs in most of the trout-producing areas of the United States and also in southeastern Canada. Recently, the virus was isolated from French trout, but the victims had been hatched from eggs obtained in North America (Wolf, 1966). IPN is typically a disease of the very young, and it usually results in high mortality. As fry grow older, severity of the infection declines and mortality is usually less; only infrequently are fingerlings (about 15 cm) killed by IPN virus. Parisot et al. (1965) reported an exceptional instance in which 25-cm (18-month-old) cutthroat trout (*Salmo clarki*) were diseased.

There are usually survivors of IPN epizootics, but some of the victims retain the virus into sexually functional maturity. In all outward appearances the adults seem to be normal, but they shed virus with feces, eggs, and sperm (Wolf et al., 1963); therefore, there is the opportunity for both contact and vertical transmission. There are, as far as we have been able to determine, no reports of experimental IPN infection of mature trout.

The immune response of teleosts to various antigens is temperature-dependent but similar in some other ways to that of higher vertebrates, and Sigel (1967) has made a recent review of the subject. Most of the antigens used in such work either have been foreign proteins such as mammalian serum

Printed in Canada (J1446)

albumin or exotic microbial forms from higher vertebrates. When bona fide fish pathogens have been used they have been administered as killed preparations such as bacterins. As an example, Krantz et al. (1964) found that trout developed agglutinin titers against a bacterin of *Aeromonas salmonicida* that equalled the titers produced by rabbits, but at 11 C, the fish required 3–4 months to reach maximal titer whereas comparable response in rabbits occurred in a month or less. Klontz et al. (1965) inoculated three fish viruses into young salmonids and reported electrophoretic, organ, and cellular changes that occurred. Precipitating and virus-neutralizing antibodies could not be found in serum from large fingerling rainbow trout (*Salmo gairdneri*) that received three sublethal inoculations of the chinook salmon virus. IPN and sockeye viruses killed the experimental hosts within 6 days, presumably before a detectable immune response could be mounted. Wolf et al. (1963) found that adult IPN carrier trout that shed much virus tended to have little or no neutralizing antibody and conversely that the individuals with high antibody titers had little or no virus. The infections had been incurred naturally; therefore, it was not possible to fix the time when IPN virus had first been encountered. Since the fish came from a hatchery with a history of IPN, it seems likely that they had contracted the disease during the fry stage.

Considering the foregoing, we inoculated feral adult rainbow trout with live IPN virus to stimulate an immune response and obtain a supply of antibody, and secondarily to observe whether or not an inoculation of live virus was capable of producing disease in such fish. The fish did not exhibit abnormal or unusual behavior changes nor did they show disease symptoms. Their immune response varied from weak to very strong, and we sought confirmation of the results and support for our conclusions in work with specific pathogen-free fish.

MATERIALS AND METHODS

Fish

Rainbow trout weighing from 570 to 1200 g were used for the principal work. The fish were taken from the stream that receives the outfall from the Leetown National Fish Hatchery and the Eastern Fish Disease Laboratory in West Virginia. There is no known reproduction of rainbow trout in the stream. The rainbow trout represented hatchery stocking and escapement from both the hatchery and laboratory. During the years that preceded this work, the hatchery had a history of IPN epizootics and the laboratory annually carried out research on this disease; accordingly, the fish had repeated opportunity for exposure to IPN virus before the research began. Prior to experimental inoculation with virus, the fish were anesthetized, bled, and individually marked by cautery. A second bleeding was made during the 12th or 13th week post-inoculation. Sera were separated and stored at −20 C.

Three-year-old rainbow trout stock at the Newcastle National Fish Hatchery, Va., had a record of freedom from IPN (Jimmy E. Camper, personal communication). They were virologically examined and found to be free of virus (K. Wolf, unpublished data). These trout were used as a source of serum from fish known to be virus-free.[1]

Two 4-year-old Bellefonte, Pa., strain brook trout (*Salvelinus fontinalis*), weighing 550 and 700 g had been reared in the laboratory, and had been examined virologically each year.

[1]Blood specimens were provided through the courtesy of Jimmy E. Camper.

Using methods capable of demonstrating known viruses of salmonids, they were shown to be free of IPN and other viruses. Prior to inoculation with virus, the fish were anesthetized and bled, and a sample of their feces taken for virological examination. Three weeks later, feces were taken again for quantitative viral assay. After 10 months the fish were bled and killed. Feces, kidney, and gonads were immediately assayed quantitatively for virus. Serum samples were stored at −20 C.

Water

Fish were held in water that supplies the Leetown National Fish Hatchery. Physical and chemical properties of this water have been reported elsewhere (Warren, 1963). Temperature during holding was 12.5 C.

Virus

IPN virus inoculum used for rainbow trout was prepared from frozen (−20 C) homogenate of viscera from infected fish (Reference IPN virus strain ATCC VR 299 was subsequently derived from the same stock.) Rainbow trout were force fed 10^6 cell culture infectious doses — 50% endpoint (ID_{50}) by intubation and a second equal dose was injected intraperitoneally. Brook trout received a single intraperitoneal injection of 10^7 ID_{50} of cell culture grown strain ATCC VR 299 IPN virus.

Cell Cultures

RTG-2 cells (Wolf and Quimby, 1962) were cultured as monolayers in static 16-mm tubes at 16–20 C. Cells were grown in Eagle's Minimal Essential Medium with 10 or 12% fetal bovine serum, 100 or 200 IU penicillin, 100 or 200 μg streptomycin, and 25 IU nystatin per milliliter.

Neutralization Test

Serum neutralization tests of virus in RTG-2 cell cultures were made by reacting a constant amount of unheated serum with an equal volume of serial 10-fold dilutions of virus. Paired pre- and post-inoculation sera were tested simultaneously and each test included a separate virus titration. Sera from the feral rainbow trout were used without dilution, but because less was available, brook trout sera were diluted 1:3.16 ($10^{-0.5}$). Sera and virus were reacted for 1 hr at 10–20 C after which 0.2 ml of each mixture was inoculated into five replicate cell cultures. Cell cultures were examined and scored for cytopathology after 5 days incubation, and 50% endpoints were determined by the Reed–Muench method (Casals, 1967). The difference in titer of pre- and post-inoculation sera, expressed as the antilogarithm, is termed the Neutralization Index. As is standard for this test, indices of less than 10 are considered negative, those from 10 to 49 are equivocal, and those over 50 are positive, i.e., indicative of infection (Casals, 1967; McKenna, 1966).

Quantification of Virus in Feces and Tissues

Materials to be quantified were suspended (feces) or homogenized (tissues) in Hanks' balanced salt solution, diluted to 10^{-1}, and then filtered through a Millipore HA (0.45 μm) membrane. Further serial 10-fold dilutions were made as deemed necessary and 0.1 ml of each dilution was inoculated into three replicate cell cultures.

RESULTS

Twenty-three rainbow trout received 2×10^6 ID_{50} of IPN virus, but none subsequently showed behavior changes or signs indicative of disease and all but one were alive after 3 months. One fish died within 24 hr of initial handling, bleeding, and marking, and its death was attributed to trauma.

Serum neutralization tests were completed for 15 fish, two-thirds of which showed a Neutralization Index greater than 50, thus indicating that they had

mounted a significant immune response and had produced what we considered to be desirable amounts of antibody (Table 1).

Among the feral rainbow trout showing a Neutralization Index of less than 50, pre-inoculation serum from three (no. 8, 14, and 15) had a neutralizing activity of from 2.6 to 3 $\log_{10}$, but the remaining two (no. 6 and 7) neither had nor developed appreciable antibody.

TABLE 1. Neutralization indices[a] of sera from feral rainbow trout adults after inoculation with 2×10^6 ID_{50} IPN virus.

Fish no.	Titer[b]			Neutralization Index
	Virus without serum	Virus + pre-inoculation serum	Virus + post-inoculation serum	
Male				
1	8.63	7.38	≤3.50	≥7,586
2	8.50	7.00	≤3.50	≥3,162
3	8.63	7.63	≤4.50	≥1,349
4	8.84	7.63	≤4.50	≥1,349
5	8.17	7.50	5.75	56
6	8.50	7.38	6.50	8
7	8.50	≥8.17	7.50	≤5
8	8.50	≥5.75	5.50	≤2
Female				
9	8.17	6.72	≤2.50	≥16,600
10	8.50	7.50	≥3.50	≥10,000
11	8.17	6.17	≤2.50	≥4,677
12	8.50	7.17	≤3.67	≥4,677
13	8.84	7.84	≤4.50	≥2,188
14	8.17	≤5.50	3.84	≤14
15	8.50	≤5.50	4.50	≤10

[a]Antilogarithm of the difference between titers of virus with pre-inoculation serum and post-inoculation serum.

[b]Expressed as exponent of $\log_{10}$ infectivity (50% endpoint) in RTG-2 cultures.

With the exception of no. 7 and possibly no. 5, pre-inoculation sera from all other fish showed viral neutralizing activity of 1 $\log_{10}$ or greater, indicating an earlier antigenic experience with IPN virus. In contrast, and supporting this finding, was the fact that 10 sera from specific pathogen-free hatchery rainbow trout brood stock not only were without neutralizing activity but actually enhanced infectivity (Table 2).

We wish to stress that most of the high post-inoculation serum neutralizing antibody levels are actually less than maximal; had additional serum been available, greater Neutralization Indices probably would have been scored.

Two brook trout adults received 10^7 ID_{50} IPN virus and showed no clinical sign of disease. Like most of the experimentally inoculated rainbow trout they

TABLE 2. Effect of normal (IPN-free) adult rainbow trout serum on IPN virus infectivity.

Titer[a]		
Without serum	With male serum	With female serum
6.50	6.83	6.37
7.75	8.00	8.25
7.75	8.00	8.50
7.75	8.25	7.83
7.75	8.25	7.50

[a]Expressed as exponent of $\log_{10}$ infectivity (50% endpoint) in RTG-2 cultures.

too developed a significant Neutralization Index (Table 3). Feces that were taken immediately prior to inoculation showed no evidence of virus, but 3 weeks later the male fish shed $10^{2.1}$ and the female $10^{3.5}$ ID_{50} of virus per milliliter of feces. Nine months later, however, the feces were again without detectable virus, and when the fish were killed virus could not be found in the two principal target tissues, the kidneys or the gonads.

TABLE 3. Neutralization Indices of sera from specific pathogen-free brook trout adults after inoculation with 10^3 ID_{50} IPN virus.

	Titer[a]		
Virus without serum	Virus + pre-inoculation serum	Virus + post-inoculation serum	Neutralization Index
6.50 — male	7.17	2.37	63,000
female	6.50	0.00	≥3,160,000

[a]Expressed as exponent of $\log_{10}$ infectivity (50% endpoint) in RTG-2 cultures.

DISCUSSION

Neither species of adult trout showed signs of disease after inoculation with live IPN virus; this is wholly in accord with the biology of the host–parasite relation as it is thus far known. The presence of virus neutralizing activity in pre-inoculation sera from the feral rainbow trout is consistent with the history of these fish in their particular environment, and one can conclude that most had sustained an earlier infection. In a population with a high incidence of carriers, Wolf et al. (1963) found that brook trout with high viral neutralizing antibody titers shed little or no virus and conversely that fish with little or no antibody shed the most virus. Accordingly, it can be postulated

that fish no. 6 and 7 were probably active carriers of IPN virus, but that fact was not determined.

As hoped, high neutralizing antibody titers had developed in the rainbow trout by the end of the 3rd month. In this respect the results were similar to those that had been obtained at comparable temperatures from brown trout (*Salmo trutta*) and brook trout in response to bacterial antigen with and without adjuvant (Krantz et al., 1964). The results differ in at least one important respect; formalin-killed cells (5×10^9 per fish) had been used as the bacterin, whereas the rainbow trout received a much smaller mass of viral antigen (2×10^6 ID_{50}). The apparent superiority of the viral antigen is due either to its establishing an infection and thereby persisting antigenically or to its stimulation of an anamnestic reaction.

As borne out by presence of virus in feces at 3 weeks post-inoculation, the two brook trout that received virus for the first time as adults actually sustained an active infection without clinical signs of disease, but it was a transient infection; they mounted a very strong immune response and virus was effectively cleared by the 10th month. In contrast, where virus is first encountered in the early fry stage, IPN virus infection can persist for years — presumably an instance of immune tolerance in which foreign antigens (IPN) are recognized as "self" by the neonatal immune system.

REFERENCES

CASALS, J. 1967. Immunological techniques for animal viruses, p. 113–198. *In* K. Maramorosch and H. Koprowski [ed.] Methods in virology. Vol. 3. Academic Press, Inc., New York.

KLONTZ, G. W., W. T. YASUTAKE, AND T. J. PARISOT. 1965. Virus diseases of the Salmonidae in western United States. III. Immunopathological aspects. Ann. N.Y. Acad. Sci. 126: 531–542.

KRANTZ, G. E., J. M. REDDECLIFF, AND C. E. HEIST. 1964. Immune response of trout to *Aeromonas salmonicida*. Part I. Development of agglutinating antibodies and protective immunity. Progressive Fish-Culturist 26: 3–10.

MCKENNA, J. M. 1966. Immunity, p. 78–118, Chap. 4. *In* J. E. Prier [ed.] Basic medical virology. Williams and Wilkins, Baltimore.

PARISOT, T. J., W. T. YASUTAKE, AND G. W. KLONTZ. 1965. Virus diseases of the Salmonidae in western United States. I. Etiology and epizootiology. Ann. N.Y. Acad. Sci. 126: 502–519.

SIGEL, M. M. 1967. Virus infection and immunity in fish, p. 301–322. *In* Gerald Berg [ed.] Transmission of viruses by the water route. Interscience Publishers (John Wiley & Sons), New York.

WARREN, J. W. 1963. Kidney disease of salmonid fishes and the analysis of hatchery waters. Progressive Fish-Culturist 25: 121–131.

WOLF, K. 1966. The fish viruses, p. 35–101, Chap. 2. *In* K. M. Smith and M. A. Lauffer, [ed.] Advances in virus research. Vol. 12. Academic Press, Inc., New York.

WOLF, K., AND M. C. QUIMBY. 1962. Established eurythermic line of fish cells *in vitro*. Science 135: 1065–1066.

WOLF, K., M. C. QUIMBY, AND A. D. BRADFORD. 1963. Egg-associated transmission of IPN virus of trouts. Virology 21: 317–321.

NOTES

Effect of Temperature on the Activity of Glyceraldehyde-3-Phosphate Dehydrogenase; a Comparison of Halibut and Rabbit Enzymes

JOHN W. FARRINGTON

Graduate School of Oceanography
University of Rhode Island, Kingston, R.I., USA

AND

DWIGHT L. BAKER

Southeastern Massachusetts Technological Institute
North Dartmouth, Mass. 02747, USA

FARRINGTON, JOHN W., AND DWIGHT L. BAKER. 1969. Effect of temperature on the activity of glyceraldehyde-3-phosphate dehydrogenase; a comparison of halibut and rabbit enzymes. J. Fish. Res. Bd. Canada 26: 2517–2521.

The halibut enzyme activity was found to be less affected by temperature change. The energy of activation of the reaction catalyzed by these two enzymes differed by a factor of 2. Both enzymes catalyzed reactions that had a constant energy of activation over the range of 5–35 C.

Received February 17, 1969

A DECREASE IN VIGOR with a decrease in the temperature of the environment is usually observed in the activity of land-based, cold-blooded animals. Crozier (1924) has shown that the creeping of an ant and the chirping of a cricket are reduced in rate by a factor of 0.5 for a 10-degree C lowering of temperature. The explanation of this phenomenon, both its qualitative and quantitative aspects, is suggested by the fact that the temperature effect on the rate of many enzyme-catalyzed reactions is such as to decrease the rate by one-half for every 10-degree C lowering of the temperature, or, as more customarily expressed, $Q_{10} = 2$ (West and Todd, 1961).

In contrast, it has long been observed that some fishes appear to be but little lethargized by low temperature (Krogh, 1916). A good example of this phenomenon is to be found in the halibut (*Hippoglossus hippoglossus*), which, when caught on hook and line in water as low as 3 C, can struggle long and vigorously, thereby demonstrating the ability to generate a rather large amount of biological energy (Bigelow and Schroeder, 1953). It is natural to wonder

Printed in Canada (J1376)

why, biochemically speaking, the halibut and other fishes appear to be so little handicapped by their cold environment.

A number of investigators have published data that indicate that some of the enzymes involved, directly or indirectly, in the locomotion of cold-water fishes are more active at low temperatures than the corresponding enzymes found in mammals. Steinbech (1949) reported that adenosine triphosphatase activity of fishes is greater than that of homeotherms at 0 C, although it is the same at 30 C. Gordon (1967), comparing the rate of oxygen uptake of muscle tissue from tuna and rat, also reported that it is the fish muscle respiration that is less depressed by lowering the temperature. Cowey (1967) demonstrated that purified glyceraldehyde-3-phosphate dehydrogenase isolated from cod or lobster is less affected in activity by lowering the temperature than the same enzyme isolated from rabbit.

We have compared the effect of temperature on the catalytic activity of glyceraldehyde-3-phosphate dehydrogenase (triosephosphate dehydrogenase = TDH), which was isolated from rabbit and halibut muscle. Our results are qualitatively similar to those reported by Cowey but show a considerably greater difference between species.

The enzyme from halibut muscle was isolated and crystallized by the procedure of Allison and Kaplan (1964). The rabbit TDH was purchased from the Sigma Chemical Co., St. Louis, Missouri.

The procedure used for the determination of dehydrogenase activity was that of Cori et al. (1948) with the following modifications: (*a*) Phosphate was used in place of arsenate and the reaction was initiated by the addition of the phosphate buffer to the assay mixture. (*b*) The change in optical density with time was automatically recorded. Reaction rates were calculated from this recording for 0.1, 0.2, and 0.3 min. These values, in μmoles NADH formed per minute, plotted against time, fell on or near a straight line. The rate of reaction at zero time (V_0) was read off the axis. The standard deviation of the determination of V_0 was 1.6%, based on 35 duplicate runs.

Substitution of phosphate for arsenate was introduced to measure the enzyme activity under more natural conditions.

Initial assays with the unmodified method of Cori et al. (1948) indicated that, at low enzyme concentration, the specific activity depended markedly upon the concentration of enzyme. It was desirable to have an assay that would give a more constant specific activity over the concentration range used in the study. Using the V_0 activity described above, we determined the specific activity of a number of TDH preparations of both species using X and 2X concentrations. The average deviation in specific activity was ±6.0%, with a range from 2 to 13%, based on six comparisons. These activities covered a range of V_0 from 18.0 to 105 μmoles NADH/min.

Three preparations of halibut TDH and two preparations of rabbit TDH were assayed for activity between 5 and 35 C. In all instances the specific activity of the enzyme preparations had decreased to varying degrees from their maximum value at the time these experiments were run. Values of V_0 in this set of data varied from a low of 12 to a high of 190 μmoles NADH/min.

For each reported value of Q_{10} (Table 1), the V_0 values in $(V_0)_{\tau+10}/(V_0)_\tau$ were obtained at the two temperatures from a single dilution of enzyme.

It seems clear from data in Table 1 that no significant difference exists, for either enzyme, between the values of Q_{10} obtained over the entire temper-

TABLE 1. Q_{10} values of rabbit and halibut triosephosphate dehydrogenase catalyzed reactions. The superscripts identify the specific enzyme preparation.

Temp range (*C*)				
5–15	10–20	15–25	20–30	25–35
		Rabbit		
2.0^2	2.2^1	2.7^2	2.0^1	1.9^2
	2.0^1	2.1^3		
	2.0^1			
	2.1^1			
	2.0^3			
		Halibut		
1.8^1	1.8^1	1.6^1	1.2^3	1.5^1
1.7^3	1.6^3	1.7^3		
1.6^3	1.4^2	1.7^3		
	1.5^3	1.2^3		

ature range. On the assumption that all values of Q_{10} for one enzyme are from a single universe, we can calculate means and standard deviations. For the rabbit enzyme reaction the mean Q_{10} is 2.1 with a standard deviation of 0.23. For the halibut enzyme reaction the mean is 1.6 and the standard deviation 0.19.

Application of the *t*-test showed that the average values of Q_{10} for the two enzymes reactions are significantly different at a level less than 1%.

The average values for Q_{10} were used to calculate the activation energy of the reaction. For the oxidative phosphorylation catalyzed by the rabbit enzyme the activation energy is 12,200 cal/mole; for the halibut enzyme catalyzed reaction it is 6700 cal/mole.

These results support the growing conviction that the ability of fishes to move vigorously in a cold environment is, in part, made possible by a system of energy-producing enzymes that reduce the activation energies of the biochemical reactions involved to unusually low values.

Discussion — Although Cowey (1967) compared rabbit muscle TDH with different marine enzymes (cod and lobster TDH) than the halibut enzyme used by us, a discussion of the difference in results from the two laboratories seems in order.

Cowey determined enzyme activities at 5, 15, 25, and 35 C and reported values for V_{max} obtained from Lineweaver–Burk plots. These values, in turn, were used to determine the activation energies from Arrhenius plots. He reports 19,000 cal/mole for the rabbit enzyme catalyzed reaction and 14,500 cal/mole for the lobster and cod enzymes.

The disparity between laboratories is seen in greater detail by a comparison of values of Q_{10}. Such values were calculated from Cowey's published activities (Table 2).

TABLE 2. Q_{10} values of rabbit, cod, and lobster triosephosphate dehydrogenase catalyzed reactions, calculated from data by Cowey (1967).

Temp (*C*)	Rabbit	Cod	Lobster
5–15	3.6	2.7	2.4
15–25	4.0	2.4	2.3
25–35	2.0	1.9	1.8

It is not possible to say, with the information at hand, whether the disparity between Cowey's results and our own is real or statistically insignificant. Cowey gives no information as to the precision of his measurements.

If the difference is real, one might look for the explanation in the different conditions under which the enzyme activity was determined. A comparison of these conditions follows with our conditions being stated first. All concentrations in the reaction mixture are given as millimolar.

(*a*) Buffer: 25 mM pyrophosphate, *p*H 8.4 — 33 mM triethanolamine, *p*H 8.6.

(*b*) NAD^+: 0.50 mM — 0.37 mM.

(*c*) Phosphate: 13 mM — 0.04–32 mM.

(*d*) D-glyceraldehyde-3-phosphate: about 0.01 mM, which is the equilibrium concentration with 2.0 mM hexose diphosphate in the presence of 0.03 mg aldolase — 0.65 mM.

(*e*) Activator-stabilizer: 3.3 mM cysteine — 0.8 mM EDTA.

Although the differences in the reaction mixtures used are considerable, it is not immediately obvious to us that the cause of the disparity lies here. We submit that further work needs to be done to resolve the question.

REFERENCES

ALLISON, W. J., AND N. O. KAPLAN. 1964. The comparative enzymology of triosephosphate dehydrogenase. J. Biol. Chem. 239: 2140–2152.

BIGELOW, HENRY B., AND WM. C. SCHROEDER. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildlife Serv. Fish. Bull. 74. p. 250, 258.

CORI, G. T., M. W. SLEIN, AND C. F. CORI. 1948. Crystallization of aldehyde-3-phosphate dehydrogenase from rabbit muscle. J. Biol. Chem. 173: 605–618.

COWEY, C. B. 1967. Comparative studies on the activity of D-glyceraldehyde-3-phosphate dehydrogenase from cold and warm-blooded animals with reference to temperature. Comp. Biochem. Physiol. 23: 969–976.

CROZIER, W. J. 1924. The critical thermal increment for the locomotion of a diplopod. J. Gen. Physiol 7: 123–136.

GORDON, M. S. 1967. Oxygen consumption of red and white muscles from tuna fishes. Science 159: 87–89.

KROGH, A. 1916. The respiratory exchanges of animals and Man. Longmans, Green and Co., London. p. 101.

STEINBECH, H. B. 1949. Temperature coefficients of muscle apyrase systems. J. Cell. Comp. Physiol. 33: 123–130.

WEST, E. S., AND W. R. TODD. 1961. Textbook of biochemistry. 3rd ed. MacMillan Co., New York. p. 409.

A Record of Hermaphroditism in Lake Whitefish, *Coregonus clupeaformis*

MAYNARD Y. CHEN

Department of Biology
University of Waterloo, Waterloo, Ont.

CHEN, MAYNARD Y. 1969. A record of hermaphroditism in lake whitefish, *Coregonus clupeaformis*. J. Fish. Res. Bd. Canada 26: 2521–2523.

A rare case of hermaphroditism in lake whitefish was observed. The ovo-testis was found in the left gonad with an unusual dorsal-ventral relationship between the two parts. The male portion contained both wide tubules densely packed with germ cells, bordering on the ovarian tissue, and narrow tubules, with fewer germ cells, in the rest of the testis. The female portion appeared to contain only atretic eggs. There was a clear demarcation between the male and female portions of the ovo-testis.

Received April 25, 1969.

HERMAPHRODITISM IN TELEOSTS is not rare although it has never been observed in lake whitefish. Since 1927 several authors have reported on examples in gobius, perch, eel, herring, salmon, and trout. Crawford (1927) observed a hermaphroditic silver salmon (*Oncorhynchus kisutch*). Turner (1931) reported an ovo-testis in the yellow perch (*Perca flavescens*). Similar observations by Gibbs (1956) in rainbow trout (*Salmo gairdneri*), Uzmann and Hesselholt (1958) in chum salmon (*Oncorhynchus keta*), and many others all lead us to believe the anomalous condition of hermaphroditism in fishes is no rarer than in other normally dioecious animals.

The author, during a study of lake whitefish in Newfoundland in 1965 and 1966, observed a rare case of hermaphroditism. The specimen (total length 323 mm, fork length 286 mm, weight 225 g) was among 261 fish sampled from

Printed in Canada (J1460)

Hogan's Pond in the vicinity of St. John's, Nfld. It had a normal right testis and an apparently normal but shortened left testis. On the ventral surface of the anterior part of the left testis there was a small but rather perfect ovary attached by the mesovarian, which extended from the membrane covering the testis. This fish, age 4+, was taken during the spawning season, i.e., late October and early November, but there was no sign as to whether the fish had spawned (percentage of maturity for the 4+ female whitefish in Hogan's Pond was calculated as 81.6%). Both ovary and testes were mature. The eggs in the ovary were in the stage of active growth. Egg diameters were between 1.4 and 1.6 mm, whereas the diameter of ripe eggs in other fish was between 2.5 and 3.0 mm.

The gonads were removed and fixed in Bouin's solution. They were later embedded in paraffin, sectioned at 5 μ and stained with H-E (hematoxylin-eosin stain). The right gonad turned out to be a normal testis with the lobules filled with germ cells in various stages of maturity.

The left gonad comprised the ovo-testis as described above. As the gross morphology revealed, this was primarily testicular tissue. The ovary constituted a piece of tissue 1-cm long on the ventral border of the testis. Although the gonads were connected by a thin piece of superficial connective tissue, the sections showed there was a clear demarcation between male and female cells (Fig. 1). The male section contained germ cells at the same level of development as those in the right testis. The section of the testis bordering on the ovarian tissue contained wide tubules which were densely packed with germ cells. In the rest of the testis, however, the tubules were narrower, and the cells were less abundant — a situation very similar to that in the right testis. There was a gonadal duct attached to the male portion and this looked like a normal vas deferens.

The female portion contained eggs in various stages of development. There were a few primary and secondary oocytes, but the great bulk of the tissue consisted of mature ovarian follicles, which were apparently undergoing absorption. An oviduct was absent.

This instance of hermaphroditism was in general unlike any mentioned in the literature for bony fishes. In superficial appearance the ovo-testis was probably most similar to the one in silver salmon reported by Crawford (1927). In both instances, the ovarian section was ventral to the testicular section. The usual pattern is an anterior-posterior relationship between the two parts. Lagler and Chin (1951) reported an abundance of connective tissue in the ovo-testis, and James (1946) reported a more than usual amount of connective tissue in the testicular portion of an ovo-testis in the largemouth bass. This was not apparent in the whitefish specimen. Most writers including James (1946), Lagler and Chin (1951), and Ross et al. (1963) mention an intermingling of male and female germ cells in the ovo-testis of bony fishes. In the present instance, however, male and female tissues were quite separated.

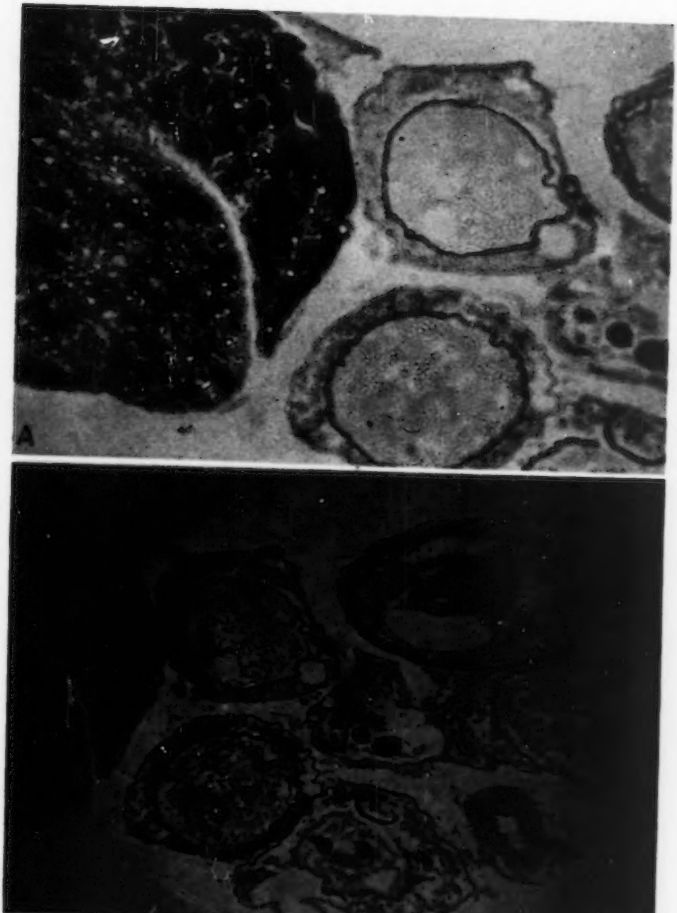

FIG. 1. Histological structures of ovo-testis of lake whitefish (*Coregonus clupeaformis*) taken from Hogan's Pond, St. John's, Nfld., on November 5, 1966. (A) ×43; (B) ×40.

Chen — J. Fish. Res. Bd. Canada.

Acknowledgments — I wish to thank Dr G. Power, Associate Professor of the Department of Biology, University of Waterloo, Waterloo, Ont., for reading the manuscript and for helpful criticism.

REFERENCES

CRAWFORD, D. R. 1927. Notice of hermaphroditism in silver salmon, *Oncorhynchus kisutch*. Copeia 163: 34.

GIBBS, E. D. 1956. A bisexual steel-head trout. Calif. Fish. Game 42: 229–231.

JAMES, M. F. 1946. Histology of gonadal changes in the bluegill, *Lepomis macrochirus* (Rafinesque) and the largemouth bass, *Huro salmoides* (Lacépède). J. Morphol. 79: 63–92.

LAGLER, KARL F., AND MARY B. CHIN. 1951. Ovo-testes in the percid fish, *Boleosoma nigrum* (Rafinesque). Copeia 1951: 99–100.

ROSS, A. J., W. T. YASUTAKE, AND G. R. WHITE. 1963. Hermaphroditism in rainbow trout. Trans. Am. Fish. Soc. 92: 313–314.

TURNER, C. L. 1931. An ovo-testes in the yellow perch (*Perca flavescens*). Science 74: 370.

UZMANN, J. R., AND M. N. HESSELHOLT. 1958. Teratological hermaphroditism in the chum salmon, *Oncorhynchus keta*. Progressive Fish-Culturist 20: 191–192.

Effect of Seawater Chilling on Landed Quality of Scallop Meat

S. VARGA AND C. M. BLACKWOOD

Department of Fisheries and Forestry of Canada
Fish Inspection Laboratory, Halifax, N.S.

VARGA, S., AND C. M. BLACKWOOD. 1969. Effect of seawater chilling on landed quality of scallop meat. J. Fish. Res. Bd. Canada 26: 2523–2526.

Landed quality of scallop (*Placopecten magellanicus* Gmelin) meat was improved by chilling in sea water prior to bagging and icing. The results suggested an increase in ice storage time of 2–9 days on trips of 14 or 15 days to Georges Bank and longer on trips to grounds where water temperatures are higher than 60 F.

Received November 25, 1968

IN RECENT YEARS the Canadian fishing effort for scallops has been extended to the area off the coast of Virginia and Carolina (ICNAF Subarea 6). The maximum meat temperature after washing in this location is as high as 77 F (LeBlanc, MS, 1966), in comparison with 63 F on the customary fishing grounds (ICNAF Subarea 4) (Thurber, MS, 1967). Canadian boats fishing in Subarea 6 during the summer and early fall have landed significant quantities of poor and reject quality scallop meat, resulting in the discontinuance of fishing in this area.

Investigations by the Department of Fisheries of Canada showed that the time required to lower the temperature of scallop meat at the center of

Printed in Canada (J1295)

a 35- to 40-lb bag to 32 F, with ice, was 18–20 hr on Georges Bank and as much as 36 hr off the coast of Virginia (Thurber, MS, 1967). The higher maximum temperatures at the Virginia coast than on Georges Bank were believed to be responsible for the low quality meat landed from Subarea 6 after the customary 14- to 15-day fishing trip. Chilling of the meat prior to bagging appeared to be a solution to the quality problem. The effectiveness of such chilling, however, had to be determined. This study had to be carried out during fishing trips to Georges Bank since the boats ceased to fish for scallops at the Virginia coast in the late summer of 1967.

Results from experiments conducted with a commercially designed unit with a Dole-type heat exchanger are reported here.

Equipment — The refrigerating equipment (Fig. 1) was designed by Consolidated Supply Co. Ltd., Halifax, N.S., and built by Dole Refrigeration Products Co., Oakville, Ont. It was installed on the scallop dragger *Charlotte & Rickey*. Evaporating plates were situated inside the

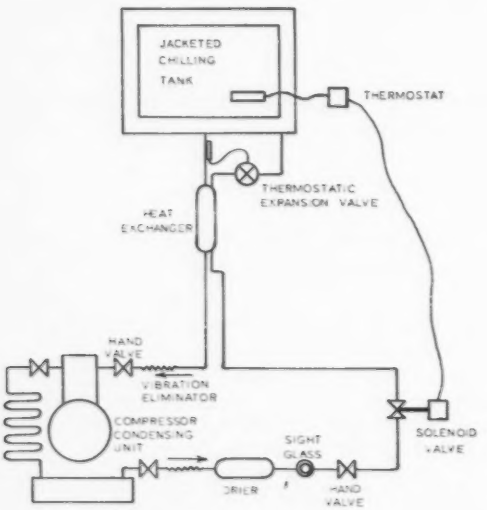

FIG. 1. Refrigeration system for chilling scallop meat.

double walls of the chilling tank and Freon 12 was used as a refrigerant. The internal dimensions of the chilling tank were 4 × 2 × 2 ft. The compressor's capacity was given as 12,000 B.T.U./hr at 15 F evaporating and 100–130 F condensing temperatures. Power was provided for the compressor by a 1.5-hp electric motor. The equipment was designed to chill 300 lbs of meat per hour to 32 F from an initial temperature of 70 F. The tank was half filled with sea water and refrigerated to 30–32 F prior to loading. The rate of loading varied from 35 to 150 lb/hr. Sea water was drained from the tank after each 6-hr watch and the tank was rinsed.

Methods — The experiment was conducted during two commercial fishing trips from Lunenburg, N.S., to Georges Bank in 1967. The first trip was made on July 7–21 and the second on October 9–24. The meat temperature after washing varied from 50 to 60 F during the trips.

For one series of daily lots for each trip, the scallop meat was washed and chilled to 30–32 F. Chilling was carried on during a 6-hr watch; after shucking and washing, the meat was continuously dumped into the immersion tank. At the end of the watch, the meat was bagged and iced in the hold. A second series of daily lots was handled as in commercial fishing. That is, the meat was washed in sea water, and bagged and iced in the hold at the end of the watch. Lots held on ice for 1 day before landing provided control samples.

At dockside a 5-lb sample was taken from each of the lots in the two series. Samples of the unchilled meat were taken as nearly as possible from the centers of the bags. All samples were placed in plastic bags and then quick-frozen in a blast freezer without delay. The frozen samples were packed in insulated coolers, transported by car to the Halifax laboratory, and stored at -10 F. The shipping required less than 2 hr and all the samples arrived at the laboratory hard frozen. Samples were examined for quality the day after the delivery.

Taste panel examinations were carried out by an experienced eight-member taste panel. Overall quality of the meat was expressed by a 10-point scoring system. The quality of a given sample was derived by averaging the scores of panel members. The assessments were carried out immediately after cooking the thawed samples at 450 F for 22 min in a pan covered with aluminum foil. Four coded samples and a known control sample were evaluated by the panel at each sitting.

For total bacteria counts, a 100-g portion from each frozen sample was aseptically placed in a sterile Waring Blendor jar. The samples were blended with twice their weight of sterile water for 2 min. The homogenates were diluted, plated in duplicate on approximately 10 ml of trypticase glucose extract agar, and incubated at 20 C for 72 hr. Colonies were counted with the Quebec colony counter. Counts from the duplicate plates were averaged, multiplied by the dilution factor, and then expressed as total counts per gram. The trypticase medium was composed of 0.5% tryptose, 0.3% beef extract, 0.1% glucose, and 1.5% agar.

The trimethylamine (TMA) concentration was determined by Dyer's (1945, 1959) picrate method. TMA was extracted with distilled water (Hoogland, 1956) rather than with trichloroacetic acid.

For statistical analysis the data from the two trips were combined to increase the degrees of freedom. Taste panel scores, total bacteria counts, and TMA values were tested for differences at $P = 0.05$ with 1 and 24 df by the two-way analysis of variance (Li, 1964).

The taste panel scores and TMA values (Hoogland, 1958) were plotted against ice storage time. The best fitting lines were calculated by the method of least squares (Li, 1964) with 23 df.

Results and discussion—The TMA values were lower and the taste panel scores higher for the chilled than for the unchilled meat (Fig. 2), the differences being significant at $P = 0.05$. The regression coefficients for TMA values and taste panel scores were both lower for chilled than for unchilled meat.

There was no significant difference between bacteria counts for the two series of samples, indicating that bacteria counts are not useful as indicators of meat quality. This observation is similar to that made by Castell et al. (1948) on cod fillets.

The lower regression coefficients for both quality indices for chilled meat indicate a lower rate of deterioration than for unchilled meat. Extrapolation of the regression lines for chilled meat indicated a 2- to 9-day extension of ice storage time. The improvement in storage time or landed quality was somewhat less than reported in an experiment on scallop meats irradiated within about 36 hr of shucking (Power et al., 1964). However, greater improvement in holding time or landed quality than obtained on trips to Georges Bank can be expected on trips to warmer waters.

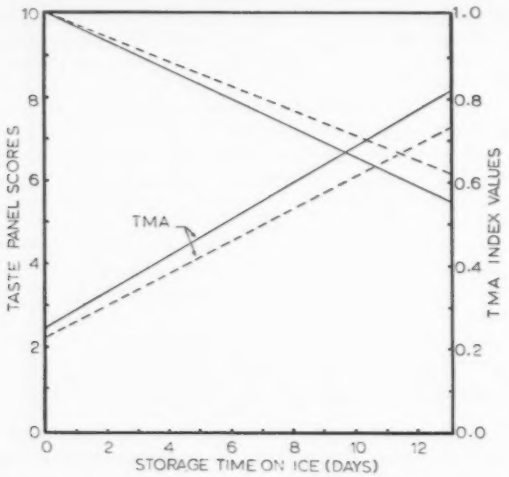

FIG. 2. Regression lines for taste panel scores and TMA values against ice storage time for chilled (broken lines) and unchilled (solid lines) scallop meat. Daily samples on each of two trips were combined for the calculations. The equations were

Meat	Taste panel scores	TMA values
Chilled	$y = 10.0 - 0.284\ x$	$y = 0.22 + 0.026\ x$
Unchilled	$y = 10.0 - 0.338\ x$	$y = 0.24 + 0.045\ x$

Acknowledgments — The authors wish to thank Dr W. J. Dyer of the Fisheries Research Board, Halifax, for criticism and advice on the manuscript. Appreciation is expressed for service on the taste panel to Messrs W. E. Anderson, A. B. Dewar, F. J. Rahey, G. Shum, J. A. Hall, and W. P. Leahy, employees of the Fish Inspection Laboratory at Halifax.

REFERENCES

CASTELL, C. H., G. W. ANDERSON, AND H. PIVNIK. 1948. Relation of bacterial counts to quality of cod fillets. J. Fish. Res. Bd. Canada 7: 378–388.

DYER, W. J. 1945. Amines in fish muscle. I. Colorimetric determination of trimethylamine as the picrate salt. J. Fish. Res. Bd. Canada 6: 351–358.
1959. Report on trimethylamine in fish. J. Assoc. Offic. Agr. Chemists 42: 292–294.

HOOGLAND, P. L. 1956. A convenient arrangement for the determination of trimethylamine in fillets. Fish. Res. Bd. Canada, Technol. Sta. Halifax Circ. 3. 10 p.
1958. Grading of fish for quality. 2. Statistical analysis of the results of experiments regarding grades and trimethylamine values. J. Fish. Res. Bd. Canada 15: 717–728.

LEBLANC, J. A. MS, 1966. Report on scallop fishing trip aboard dragger "Maria D." Dept. Fish. Canada Unpublished Rept. 6 p.

LI, J. C. R. 1964. Statistical inference I. Edward Brothers Inc., Ann Arbor, Michigan. 658 p.

POWER, H. E., D. I. FRASER, W. NEAL, W. J. DYER, C. H. CASTELL, AND H. C. FREEMAN. 1964. Use of gamma radiation for the preservation of scallop meat. J. Fish. Res. Bd. Canada 21: 813–826.

THURBER, L. K. MS, 1967. Report on scallop fishing trip aboard dragger "Charlotte & Rickey." Dept. Fish. Canada Unpublished Rept. 5 p.

Deep-Sea Line Fishing off British Columbia

NEIL BOURNE AND M. A. POPE[1]

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

BOURNE, NEIL, AND M. A. POPE. 1969. Deep-sea line fishing off British Columbia. J. Fish. Res. Bd. Canada 26: 2527–2531.

Deepwater longline sets were made in 1830–2830 m off the British Columbia coast between 50°31′–54°32′ N and 129°00′–134°35′ W in 1965. A total of 85 individual fishes were caught, which included five species.

Received May 2, 1969

FEW ATTEMPTS have been made to sample the deepwater demersal fish populations off the British Columbia coast. (The term "deepwater" as used here means depths of 1000 m (545 fath) or greater.) In 1888, the United States Fisheries Commission steamer *Albatross* made three hauls with a bottom drag off the Queen Charlotte Islands and Vancouver Island, one of these in 876 fath (1600 m) (Bean, 1890). In 1890, another haul was made off Moresby Island (Queen Charlotte Islands) in 1588 fath (2900 m) (Gilbert, 1896).

Numerous tows were made by the International Pacific Halibut Commission from 1926 to 1932 during surveys for halibut eggs and larvae off the British Columbia coast. Most tows were pelagic and at depths of less than 1000 m, but a few were in depths from 1000 to 1500 m. Numerous species of fish were caught during this work, including 11 new species and 3 new genera, all bathypelagic (Chapman, 1939, 1940).

In 1964, a program was initiated at the Fisheries Research Board of Canada Biological Station, Nanaimo, B.C., to survey the bottom invertebrates off the coast of British Columbia to depths of 2750 m (1500 fath). Several species of fish were captured in the various types of bottom sampling drags used during this work. Forester (1964) pointed out that line fishing is an excellent method for taking larger midwater or abyssal fish. During the bottom dragging program in 1965, a longline was set at selected stations to sample the larger deepwater demersal fishes off British Columbia, the results of which are reported here.

Methods — The fishing method used was a modification of that described by Forester (1964). The groundline was 550 m (300 fath) of 4.75-mm ($\frac{3}{16}$-inch) diam tarred nylon; the buoy line, 3650 m (2000 fath) of 9.5-mm ($\frac{3}{8}$-inch) diam braided, coarse filament polypropylene.

One hundred hooks were attached at approximately 5.5-m (3-fath) intervals along the groundline. The gangions were 75 cm in length and were either the standard halibut braided nylon type or the braided nylon with a leader. They were attached to the groundline with ordinary halibut snaps (Fig. 1E). If a leader was used, a small stainless steel swivel was placed between the gangion and the leader.

[1]Present address (M.A.P.): 2166 Kings Avenue, Vancouver, B.C.

Printed in Canada (J1466)

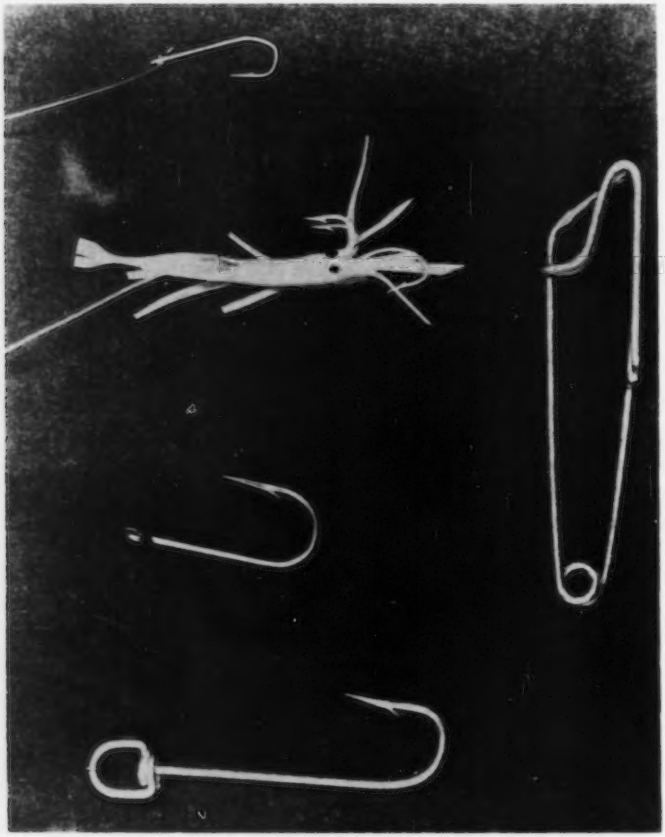

FIG. 1. Types of hooks used on longline and halibut snap: (A) Japanese longline salmon hook; (B) luminous artificial shrimp; (C) coho salmon hook; (D) conger eel hook; (E) halibut snap.

Six combinations of hooks and gangions were used for each set (Fig. 1): (*a*) 10 luminous artificial shrimps with triple hooks on 38-cm braided nylon gangions and 30-cm nylon monofilament leaders; (*b*) 30 conger eel hooks on 75-cm braided nylon gangions (Forester, 1964); (*c*) 20 conger eel hooks on 38-cm braided nylon gangions and 30-cm wire leaders; (*d*) 15 coho salmon hooks on 38-cm braided nylon gangions and 30-cm nylon monofilament leaders, 32 kg test; (*e*) 15 coho salmon hooks on 38-cm braided nylon gangions and 30-cm wire leaders; (*f*) 10 Japanese salmon longline hooks on 38-cm braided nylon gangions and 30-cm nylon monofilament leaders, 32 kg test.

To eliminate possible differences in fishing efficiency because of position on the groundline, the hooks were arranged in 5 groups of 20. Each group consisted of the following: 2 luminous artificial shrimps; 6 conger eel hooks on 75-cm braided nylon gangions; 4 conger eel hooks with 30-cm wire leaders; 3 coho salmon hooks with monofilament leaders; 3 coho salmon hooks with wire leaders; and 2 Japanese salmon longline hooks.

In most instances the hooks were baited with whole or half portions of squid (*Loligo opalescens* Berry). In one set, whole salted anchovies (*Engraulis mordax* Girard) were used on

the Japanese salmon longline hooks. This was discontinued because the hooks baited with anchovies caught nothing. It was felt the anchovies were so fragile that the hooks had lost the bait by the time they reached the bottom. The artificial shrimp hooks were not baited.

The ship was allowed to drift as the line was being set. A 13.5-kg trawl anchor was attached to one end of the groundline, and the gangions were snapped on as the line was paid out. The buoy line was attached to the other end of the groundline by a large shackle, whose function is described below. The entire buoy line was then run out, and a spot buoy and floats attached to the upper end.

A major difficulty in setting deepwater longlines is to ensure that the groundline does not end up in a heap on the bottom (Forester, 1964). To prevent this, a method was used similar to that described by Forester. When the amount of buoy line paid out was about two-thirds of the depth of water, a messenger was attached to the line and released. The messenger was stopped at the shackle joining the buoy and ground lines and because of its weight caused the buoy line to sink vertically, thereby stretching the groundline along the bottom. The messenger consisted of a 35-cm piece of 25-mm diam polyethylene pipe with two 13.5-kg cannon balls attached to it.

Each set was of 2-hr duration and all sets were made at night. The line was hauled in by a mechanical line-hauler and both buoy and ground lines were wound onto a single drum.

Five sets, which ranged in depth from 1000 to 2830 m, were made in an area from Cape Scott on the northern tip of Vancouver Island to Dixon Entrance, just north of the Queen Charlotte Islands. In set 3, a 20-m riser with a float and five conger eel hooks was attached to the anchor to determine whether some fish might be feeding just off the bottom.

When the catch was landed the number of fish on each type of hook was recorded.

Results — The results of the five sets are summarized in Table 1.

Fish were caught at each set, a total of 85 individuals, which included five species. The largest catch was 26 fish from set 1; the smallest catch, 9 fish from set 3, the deepest set.

Rattails (*Coryphaenoides*) were the most common fish caught and comprised 47.6% of the total catch. Two species were caught, *C. acrolepsis* Bean, and *C. pectoralis* Gilbert, the former being much more common. The latter species was not recorded by Clemens and Wilby (1961).

A total of nine rougheye rockfish (*Sebastodes aleutianus* Jordan and Evermann) were taken. Two specimens were caught in the deepest set in 2830 m, which is considerably deeper than the maximum depth of 455 m (250 fath) reported by Phillips (1957).

Two specimens of longfin cod (*Antimora rostrata* Gunther) were caught in the first set in 1830 m. The eight specimens of sablefish (*Anoplopoma fimbria* Pallas) in set 4 were caught in 1000–1100 m, which is the same as the maximum depth recorded by Alverson et al. (1964).

The data on catch per hook type indicate some differences in fishing performance. Coho hooks (with both wire and monofilament leaders) were slightly more efficient than conger eel hooks and much more so than the Japanese salmon longline and artificial shrimp hooks.

Discussion — It is presumed that the fish were caught on, rather than just off the bottom. Some evidence for this is seen in set 3 where no fish were caught on the 20-m riser. It is possible that some specimens could have been caught well off the bottom as the line was being set or hauled.

TABLE 1. Results of deepwater line fishing off British Columbia from the *G. B. Reed*, August 1965.

Set no.	Depth (*m*)	Catch: No. fish caught on each type of hook: Luminous shrimp	Conger eel	Conger eel and wire	Coho and monofilament	Coho and wire	Japanese salmon	Types and no. fish caught	Species identified: *Coryphaenoides acrolepsis*	*Coryphaenoides pectoralis*	*Antimora rostrata*	*Sebastodes aleutianus*	*Anoplopoma fimbria*	Unidentified species: *Coryphaenoides*	Position: Lat	Long	Date	Remarks
1	1830	–	8	7	4	7	–	24 rattails; 2 longfin cod	9	1	2	–	–	14	50°31′	129°00′	Set Aug. 3 Hauled Aug. 4	–
2	1830	1	4	4	4	6	2	19 rattails; 2 rougheye rockfish	7	–	–	2	–	12	51 43 30″	131 28	Aug. 5	Bait missing on 7 hooks; one hook missing
3	2830	–	6	1	1	1	–	7 rattails; 2 rougheye rockfish	7	–	–	2	–	–	51 05	132 22	Aug. 9	Bait missing on 2 hooks; one hook missing; no catch on 20-m riser on anchor
4	1000–1100	–	10	2	3	3	–	10 rattails; 8 sablefish	9	1	–	–	8	–	53 01	132 55	Aug. 10	Lost anchor and 10 hooks
5	2000	1	3	3	3	1	–	6 rattails; 5 rougheye rockfish	6	–	–	5	–	–	54 32	134 35	Aug. 11	Bait missing on 2 hooks

Although only five species of fish were landed, moderate numbers of fish were caught considering the relatively small number of hooks used. The smallest catch was nine fish, the largest 26. No blank hauls were made. The catches might have been higher if more efficient hooks had been used in place of the artificial shrimp and Japanese longline hooks. Apparently the fish were attracted only to baited hooks since the unbaited artificial shrimp hooks caught very little. It is probable that more fish were hooked than landed since some hooks were missing in four of the sets. A greater range in size of hooks, lures, type of bait, and length of time the hooks are on the bottom might produce different results and might provide a greater variety of species of fish than we observed.

Acknowledgments — Part of the fish collection was subsequently deposited in the National Museum, Ottawa, and part in the Institute of Fisheries at the University of British Columbia, Vancouver, B.C. Those fish deposited in the National Museum have been identified by Dr D. E. McAllister to whom we wish to express our thanks.

REFERENCES

ALVERSON, D. L., A. T. PRUTER, AND L. L. RONHOLT. 1964. A study of demersal fishes and fisheries of the northeastern Pacific Ocean. H. R. MacMillan lectures in fisheries, Univ. British Columbia, Inst. Fish. 190 p.

BEAN, T. H. 1890. New fishes collected off the coast of Alaska and the adjacent region southward. Proc. U.S. Natl. Museum 13: 37–45.

CHAPMAN, W. M. 1939. Eleven new species and three new genera of oceanic fishes collected by the International Fisheries Commission from the northeastern Pacific. Proc. U.S. Natl. Museum 86: 501–542.

1940. Oceanic fishes from the northeastern Pacific Ocean. Occas. Papers, B.C. Provincial Museum 2. 40 p.

CLEMENS, W. A., AND G. V. WILBY. 1961. Fishes of the Pacific Coast of Canada. 2nd ed. Bull. Fish. Res. Bd. Canada 68. 443 p.

FORESTER, G. R. 1964. Line-fishing on the continental slope. J. Marine Biol. Assoc. U.K., 44: 277–284.

GILBERT, C. H. 1896. The ichthyological collections of the steamer "Albatross" during the years 1890 and 1891. Rept. U.S. Fish Comm. (1893) 19: 393–476.

PHILLIPS, J. B. 1957. A review of the rockfishes of California (Family Scorpaenidae). Calif. Dept. Fish Game, Fish. Bull. 104. 158 p.

Polymorphic Serum Esterases in Two Species of Freshwater Fishes

O. L. NYMAN

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

NYMAN, O. L. 1969. Polymorphic serum esterases in two species of freshwater fishes. J. Fish. Res. Bd. Canada 26: 2532–2534.

Two simple, genetically determined serum esterase polymorphisms, suited for population analysis, are described in two species of freshwater fishes, white bream (*Abramis blicca* B.) and ruff (*Acerina cernua* L.). In both instances the synthesis of the enzyme bands was found to be controlled by two allelic codominant genes, which also in both instances were kept in equilibrium. Gene frequency data are presented from one population of each species.

Received May 6, 1969

SINCE MOST MORPHOLOGICAL characteristics in fish are subject to environmentally determined modifications, population separations and taxonomic problems at the subspecific level are very difficult. With the recent development of the various electrophoretic methods numerous genetic variants of proteins have been reported. The frequencies of these variants are useful for separating populations and solving taxonomic problems. A few examples from the abundant literature may be cited. Various populations and subspecies of cod (*Gadus morhua* L.) have been reported (Sick, 1965; Møller, 1968); other significant contributions were added by Sick et al. (1967) on the systematic relationship of European and American eels (*Anguilla vulgaris* and *A. rostrata*) and by Hodgins et al. (1969) on lactate dehydrogenase heterogeneity on populations of sockeye salmon (*Oncorhynchus nerka*).

In recent years increased attention has been given to polymorphisms in the serum esterases of fishes, these enzymes being usually less complex than haemoglobins, transferrins, and dehydrogenases, besides being less affected by ontogeny and storage. Simple polymorphisms with only two or three allelic codominant genes involved have been reported (Nyman, 1965, 1967; Koehn and Rasmussen, 1967; Koehn and Johnson, 1967; de Ligny, 1968; Fujino and Kang, 1968; Nyman and Westin, 1969) and they have served as an aid in population separations and other systematic and evolutionary problems.

This report presents evidence that two additional species of fishes, ruff (*Acerina cernua* L.) and white bream (*Abramis blicca* B.), have simple two-allele serum esterase polymorphisms that may be employed in population investigations.

Materials and methods — Fifty specimens of ruff (*A. cernua* L.: Percidae) and 28 of white bream (*A. blicca* B.: Cyprinidae) were collected by gillnetting in Lake Mälaren, off the Institute

Printed in Canada (J1471)

of Freshwater Research, Drottningholm (Sweden), in the summer of 1967. Blood was sampled by cardiac puncture and withdrawn in heparinized glass capillaries. It was immediately transferred to the test tubes of the Beckman Microfuge 152 and spun for 30 sec after which the serum was collected and frozen. When serum was kept below −10 C the zymograms were still detectable after 1 year's storage. The electrophoretic procedure is described elsewhere (Nyman, 1967). It consisted of horizontal starch-gel electrophoresis; the buffer by Ashton and Braden (1961) was employed, and α-naphthyl acetate combined with the diazonium dye coupler Fast Red TR Sigma was used for substrate. Optimal enzyme activity was reached within 10 min.

Results and discussion — In both ruff and white bream three phenotypically different patterns of serum esterase are believed to occur (Fig. 1). However, no Est F/Est F phenotype was observed in the white bream sample; because of

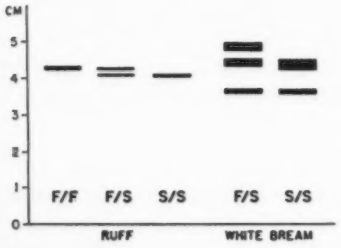

FIG. 1. Serum esterase polymorphisms in ruff and white bream. F (fast) and S (slow) designate the alleles responsible for the synthesis of the esterase zones. F and S are different genes in the two species. The horizontal line represents the starting point of electrophoresis, the vertical line shows the distance of anodic migration.

the low gene frequency only one such phenotype is expected in 125 specimens. Each band of esterase activity represents one type of subunit, which may be the product of a single allele at a locus, either the two homozygous patterns produced by homologous alleles or the heterozygous pattern representing the combination of both allelic genes. If "Est" stands for esterase and "S" and "F" for the slow and fast zones, respectively, the patterns may be termed Est S/Est S and Est F/Est F for the homozygotes and Est F/Est S for the heterozygote. The polymorphism in ruff serum esterase was described earlier (Nyman, 1965), but an insufficient number of specimens prevented a test of the generally accepted theory of the inheritance mechanism. In the same paper, the white bream esterase was described as monomorphic due to the low frequency of the Est F allele. The phenotype distribution of the present samples according to the Hardy–Weinberg law (Table 1) is consistent with

TABLE 1. Gene frequency data of samples of ruff and white bream collected from Lake Mälaren, Sweden.

	Ruff (*Acerina cernua*)	White bream (*Abramis blicca*)
Genotype		
Est F/Est F	9(8.4)[a]	0(0.2)
Est F/Est S	23(24.2)	5(4.6)
Est S/Est S	18(17.4)	23(23.2)
Total collected	50	28
p(F)	0.41(0.31–0.51)[b]	0.09(0.01–0.17)
Chi-square (1 df)	0.123	0.236
Probability of a greater value	0.70–0.80	0.50–0.70

[a]Observed frequency (expected frequency in parentheses).
[b]95% confidence limits in parentheses.

the above hypothesis that the enzymes involved are under the control of two allelic codominant genes at one locus and that the populations are in equilibrium.

Because each sample consists only of a small number of specimens from the sampled populations, it is evident that deviations from the real gene frequencies may occur. Therefore it may be practical also to show the confidence intervals since they take into consideration the number of specimens sampled. The 95% confidence interval was determined from the expression

$$p(\mathrm{F})_{\mathrm{obs}} \pm 2\sqrt{\frac{p(\mathrm{F})_{\mathrm{obs}}\,[1-p(\mathrm{F})_{\mathrm{obs}}]}{2n}}$$

where $p(\mathrm{F})_{\mathrm{obs}}$ is the observed frequency of the Est F allele and n is the number of specimens in the sample.

A heterogeneity chi-square test of the white bream sample indicated no difference in gene frequencies between the sexes or in variation with age (size).

REFERENCES

ASHTON, G. C., AND A. W. H. BRADEN. 1961. Serum β-globulin polymorphism in mice. Australian J. Exptl. Biol. Med. Sci. 14: 248–253.

FUJINO, K., AND T. KANG. 1968. Serum esterase groups of Pacific and Atlantic tunas. Copeia 1968: 56–63.

HODGINS, H. O., W. E. AMES, AND F. M. UTTER. 1969. Variants of lactate dehydrogenase isozymes in sera of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 26: 15–19.

KOEHN, R. K., AND D. W. JOHNSON. 1967. Serum transferrin and serum esterase polymorphisms in an introduced population of the bigmouth buffalofish, *Ictiobus cyprinellus*. Copeia 1967: 805–808.

KOEHN, R. K., AND D. I. RASMUSSEN. 1967. Polymorphic and monomorphic serum esterase heterogeneity in catostomid fish populations. Biochem. Genet. 1: 131–144.

LIGNY, W. DE. 1968. Polymorphism of plasma esterases in flounder and plaice. Genet. Res. Cambridge. 11: 179–189.

MØLLER, D. 1968. Genetic diversity in spawning cod along the Norwegian coast. Hereditas 60: 1–32.

NYMAN, O. L. 1965. Inter and intraspecific variations of proteins in fishes. Ann. Acad. Reg. Sci. Upsalien. 9: 1–18.

1967. Protein variations in *Salmonidae*. Rept. Inst. Freshwater Res., Drottningholm 47: 5–38.

NYMAN, O. L., AND L. WESTIN. 1969. Blood protein systematics of *Cottidae* in the Baltic drainage area. Rept. Inst. Freshwater Res., Drottningholm 49: 164–174.

SICK, K. 1965. Haemoglobin polymorphism of the cod in the Baltic and the Danish Belt Sea. Hereditas 54: 19–48.

SICK, K., E. BAHN, O. FRYDENBERG, J. T. NIELSEN, AND D. VON WETTSTEIN. 1967. Haemoglobin polymorphism of the American freshwater eel *Anguilla*. Nature 214: 1141–1142.

Kidney Disease in Juvenile Atlantic Salmon (*Salmo salar*) in the Margaree River

J. H. C. PIPPY[1]

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

PIPPY, J. H. C. 1969. Kidney disease in juvenile Atlantic salmon (*Salmo salar*) in the Margaree River. J. Fish. Res. Bd. Canada 26: 2535–2537.

Bacterial kidney disease was presumptively identified in each of 25 hatchery-reared juvenile salmon (*Salmo salar*) but in only 2 of 235 wild juveniles in the Margaree River system. Apparently spread of disease from the hatchery to wild salmon in the river is very gradual.

Received February 7, 1969

BACTERIAL KIDNEY DISEASE has been present among juvenile salmon (*Salmo salar*) at the Canadian Government Fish Hatchery on the Margaree River (Fig. 1) for some years. Since water flowing from the hatchery undoubtedly carries pathogens into the Margaree River system, the possibility that the disease has spread to wild juvenile salmon in the system was investigated.

A resumé of our present knowledge of kidney disease in fingerling to adult salmonid fishes in hatcheries is given by Wolf (1966). The causative organism is a Gram-positive diplobacillus which probably belongs to the *Corynebacterium* spp. Smith (1964) estimated that two-thirds of all adult salmon mortalities in the lower reaches of the Aberdeenshire Dee, Scotland, in 1958, were associated with the disease, indicating the disease can also affect fish in nature. The effect of bacterial kidney disease on survival of Atlantic salmon in the sea is not known, although another kidney disease, caused by the protozoan *Chloromyxium majori*, has been associated with negligible returns of hatchery-reared chinook salmon (Burrows, 1968). Establishment of the relative incidence of bacterial kidney disease in hatchery-reared and wild Atlantic salmon in the Margaree River system will provide a basis for future comparison of tagging returns of salmon from these two sources.

Methods — Salmon parr have been collected annually as part of a Fisheries Research Board of Canada field program on the Margaree River since 1957. Fifteen to twenty parr, preserved in 10% formalin, from each sampling station of the 1965 and 1967 surveys and 25 smolts from the hatchery in 1967 were examined (Fig. 1). The ventral surfaces of the kidneys were examined macroscopically. Stained (Gram) smears of all grossly abnormal kidneys and an additional five normal kidneys in each sample were examined microscopically (1250×). The presence of Gram-positive diplobacilli in a kidney showing the gross symptoms described by Smith (1964) and Wolf (1966) was used to presumptively identify bacterial kidney disease.

---

[1]Present address: Fisheries Research Board of Canada, Biological Station, St. John's, Nfld.

Printed in Canada (J1366)

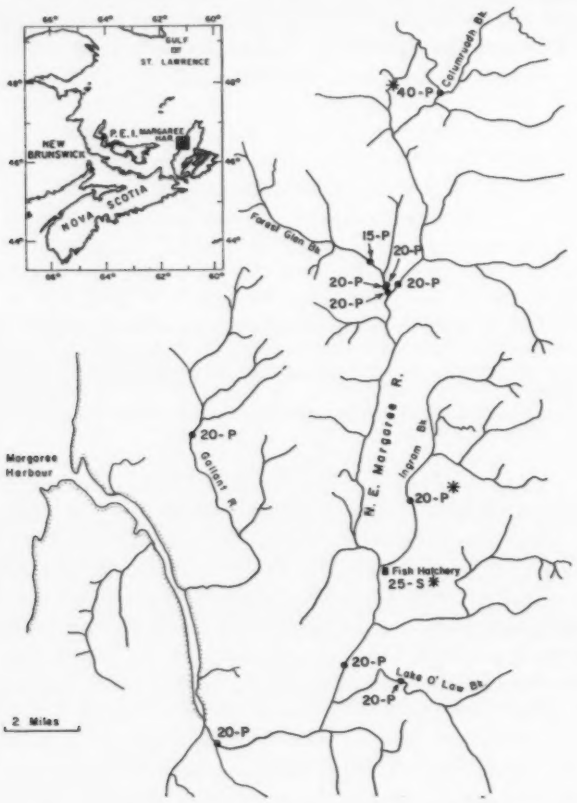

FIG. 1. Sampling stations in the Northeast Margaree River system and sites of infection. Number = sample size; P = parr, S = smolt; * indicates presence of kidney disease.

Results — Severe infection by kidney disease was presumptively identified in each of the smolts from the hatchery. This indicates a high incidence of disease at the hatchery.

A total of 235 wild parr was examined (Fig. 1) from the Margaree River system. Numerous Gram-positive diplobacilli (Fig. 2) were found in a 6-mm diam grey–white mass in the posterior third of the kidney of one parr. This fish was taken at the mouth of Calumruadh Brook. A second infected parr was taken above the fish hatchery in Ingram Brook. Only five diplobacilli were observed in this fish and the kidney was only slightly discoloured. The bacilli from these fish were from 0.4 to 0.6 μ long (mean of 20 specimens = 0.5 μ).

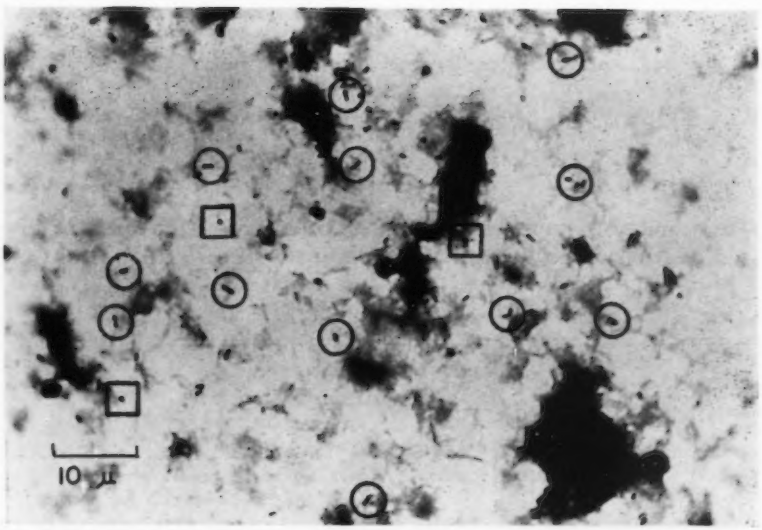

FIG. 2. Gram-positive diplobacilli from the kidney of a smolt from Calumruadh Brook (Fig. 1). Bacteria in circles; kidney granules in squares.

Discussion — It might have been expected that the incidence of kidney disease among parr in Ingram Brook would be higher than in other areas because of the greater chance of contact with bacteria from the hatchery. This was not the case. Also, the absence of the disease in samples taken below the hatchery indicates the disease is not abundant in parr from this area. However, the presence of one infected fish at the mouth of Calumruadh Brook, about 14 miles upstream from Ingram Brook, suggests that the disease may be present in fish some distance from the hatchery. These data suggest a very gradual spread of the disease from the hatchery to wild salmon in the river.

REFERENCES

BURROWS, R. E. 1968. Influence of fingerling quality on adult salmon survival. Am. Fish. Soc. 98th Ann. Meeting, Sept. 9–11, 1968, Abstr. 22.

SMITH, I. W. 1964. The occurrence and pathology of Dee disease. Dept. Agr. Fish. Scot., Freshwater Salmon Fish. Res. 34. 12 p.

WOLF, K. 1966. Bacterial kidney disease of salmonid fishes. U.S. Dept. Interior, Fish. Disease Leaflet 8. 4 p.

Meristic Analyses of Atlantic Mackerel, *Scomber scombrus*, from the North American Coastal Populations[1]

K. T. MACKAY AND E. T. GARSIDE

Department of Biology
Dalhousie University, Halifax, N.S.

MACKAY, K. T., AND E. T. GARSIDE. 1969. Meristic analyses of Atlantic mackerel, *Scomber scombrus*, from the North American coastal populations. J. Fish. Res. Bd. Canada 26: 2537–2540.

Mean counts of vertebrae, of anal and soft dorsal fin rays, and of peduncular finlets were identical in samples of Atlantic mackerel, *Scomber scombrus*, from the northern and southern breeding populations in the northwest Atlantic Ocean. This information, together with growth rates and biochemical evidence from the literature, suggests that although the populations occupy separate spawning regions there is sufficient exchange of individuals at other seasons to maintain considerable genetic continuity.

Received November 20, 1968

IN THE COURSE of a population study (MacKay, MS, 1967) of the Atlantic mackerel, *Scomber scombrus* Linnaeus, certain meristic characters were examined to determine whether numerical differences occur in various parts of the geographic range. Such differences would be concrete evidence in support of the suggestion of Sette (1950) that two populations occur along the coasts of northeastern United States of America and maritime Canada.

[1]Based on a thesis submitted by K.T.M. in partial fulfillment of the requirements for the degree of Master of Science from Dalhousie University, Halifax, N.S.

Printed in Canada (J1290)

Counts were made of total vertebral number, numbers of rays in the second dorsal fin and of soft rays in the anal fin, and numbers of peduncular finlets in several samples of mackerel that were collected from fishermens' catches at several ports. These locations were grouped into three regions (Table 1, 2). Counts of vertebral series were taken from prepared skeletons or radiographs. All vertebrae except the urostyle were counted. Rays and finlets were counted in these preparations and in several other samples from which vertebral counts were not taken. Differentiation of the second dorsal fin and the succeeding series of finlets appeared incomplete in some specimens so that the fin terminated with a partially detached posterior lobe. This structure was not counted as a finlet.

Results and discussion — A vertebral count of 30 was recorded in 241 of 242 specimens collected along the east coast of Nova Scotia; the lone variant had 29 vertebrae. Counts of 54 specimens from the more southern part of the *summer* range, at Provincetown, Massachusetts, were essentially the same with 52 specimens having 30 vertebrae and the remaining two having 28 and 29. These mean numbers of vertebrae, 30.0, agree with the mean reported by Bergeron (MS, 1961) but are each one unit lower than that given by Matsui (1967), whose samples were smaller but from more widely distributed sites. This difference of one unit suggests that the urostyle was included in enumeration in the latter study.

Counts of the rays of the second dorsal fin (Table 1) conformed in mean values, modalities, and ranges to those reported for this species by Bergeron (MS, 1961), Bigelow and Schroeder (1953), and Leim and Scott (1966). The mean number of soft anal rays (Table 1) was almost one greater than the figure of 11 given by Leim and Scott (1966). The numbers of peduncular finlets (Table 2) agreed with those reported by these several authors.

Sette (1950) separated the Atlantic mackerel along the east coast of middle North America into northern and southern "contingents" or populations largely on the basis of differential size-composition of year-classes. MacKay (MS, 1967), using results obtained from rates of growth and electrophoretic analysis of several systems of enzymes, concluded that mackerel from northern and southern regions of the summer range are not distinct in these respects. The present results, showing virtually complete coincidence in numerical comparisons of several meristic characters, perhaps give further support to the proposition that, although there are two spatially discrete breeding systems, there is sufficient desultory vagrancy to provide the necessary exchange of genetic material between the populations to maintain relatively stable characteristics.

We note in passing, however, that numerical stability in meristic characters among populations of the same species should not be equated with genetic stability a priori. The possible modifying effects of various levels of environmental variables, especially temperature, on meristic numbers should always be considered (Garside, 1966). Bigelow and Schroeder (1953) indicate that

TABLE 1. Frequency distributions and mean counts of rays of second dorsal fin and anal fin in samples of Atlantic mackerel collected from several sites along eastern coasts of southern Canada and northern United States in the summers of 1965 and 1966.

Sampling locations	Year	No. fin rays						Sample size	Mean count
		9	10	11	12	13	14		
Second dorsal fin									
Nova Scotia, Atlantic Coast	1965		20	128	284	16		448	11.66±0.06[a]
Gulf of St. Lawrence	1965		9	58	215	18		301	11.81±0.06
Provincetown, Massachusetts	1966	1	4	19	120	10		154	11.87±0.10
Anal fin									
Nova Scotia, Atlantic Coast	1965		9	97	327	14		447	11.77±0.06
Gulf of St. Lawrence	1965		6	46	237	9	1	299	11.84±0.07
Provincetown, Massachusetts	1966		3	3	43	5		54	11.92±0.10

[a]Two standard errors.

TABLE 2. Frequency distributions and mean counts of peduncular finlets in samples of Atlantic mackerel collected from several sites along eastern coasts of southern Canada and northern United States in the summers of 1965 and 1966.

Sampling locations	Year	Finlet	No. finlets			Sample size	Mean count
			4	5	6		
Nova Scotia, Atlantic coast	1965	Dorsal	6	187	7	200	5.0±0.02[a]
		Anal	5	195	–	200	5.0±0.02
Gulf of St. Lawrence	1965	Dorsal	12	281	6	299	5.0±0.02
		Anal	12	282	6	300	5.0±0.02
Provincetown, Massachusetts	1966	Dorsal	2	96	2	100	5.0±0.02
		Anal	6	92	2	100	5.0±0.02

[a]Two standard errors.

thermal conditions during July, the peak of the spawning season in the Gulf of St. Lawrence, are approximately those at the peaks of spawning in the Gulf of Maine in early June and in Chesapeake Bay in late April. Therefore, the bulk of the individuals through various regions of the breeding range apparently pass their embryonal phase in rather similar conditions. This situation also could contribute to numerical stability of such structures, through the geographic range.

Acknowledgments — We are indebted to S. N. Tibbo, Fisheries Research Board of Canada, St. Andrews, N. B., for assistance in several forms. Financial assistance was derived from the Fisheries Research Board of Canada and from the National Research Council through an operating grant to the associate author.

REFERENCES

BERGERON, J. MS, 1961. Rapport preliminaire des travaux sur la biologie de Maquereau (*Scomber scombrus* L.) du golfe Saint-Laurent. Fish. Res. Bd. Canada, Rappt. Ann. 1960, Sta. Biol. Marine, Grande-Rivière, Que. p. 77–85.

BIGELOW, H. B., and W. C. SCHROEDER. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildlife Serv. Fish Bull. 74. 577 p.

GARSIDE, E. T. 1966. Developmental rate and vertebral number in salmonids. J. Fish. Res. Bd. Canada 23: 1537–1551.

LEIM, A. H., and W. B. SCOTT. 1966. Fishes of the Atlantic Coast of Canada. Bull. Fish. Res. Bd. Canada 155. 485 p.

MACKAY, K. T. MS, 1967. An ecological study of mackerel *Scomber scombrus* Linnaeus in the coastal waters of Canada. Fish. Res. Bd. Canada Tech. Rept. 31. 127 p.

MATSUI, T. 1967. Review of the mackerel genera *Scomber* and *Rastrelliger* with description of a new species of *Rastrelliger*. Copeia 1967: 71–83.

SETTE, O. E. 1950. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 2. Migration and habits. U.S. Fish Wildlife Serv. Bull. 49: 251–358.

Recent Articles on FRB Investigations Published in Other Periodicals

Studies No.

1327. MERCER, M. C. 1968. A synopsis of the recent Cephalopoda of Canada. Proc. Symp. Mollusca 1: 265–276.

1328. BUTLER, T. H. 1968. The shrimp fishery of British Columbia. FAO Fish. Rept. 2: 521–526.

1329. IDLER, D. R., G. B. SANGALANG, AND A. KANAZAWA. 1969. Steroid desmolase in gonads of a marine invertebrate, *Placopecten magellanicus* Gmelin. Gen. Comp. Endocrinol. 12: 222–230.

1330. ADDISON, R. F., AND R. G. ACKMAN. 1969. Separation of triglycerides and free fatty acids on Sephadex LH-20. Anal. Biochem. 28: 515–522.

1331. ACKMAN, R. G. 1969. Temperature effects in the calculation of equivalent chain length values for multiple-branched fatty acid esters and ketones on polar and non-polar open tubular columns. J. Chromatog. 42: 170–175.

1332. MUNDIE, J. H. 1969. Ecological implications of the diet of juvenile coho in streams. H. R. MacMillan Lectures in Fisheries, Symp. Salmon Trout Streams 1968: 135–152.

1333. BAMS, R. A. 1969. Adaptations of sockeye salmon associated with incubation in stream gravels. H. R. MacMillan Lectures in Fisheries, Symp. Salmon Trout Streams 1968: 71–87.

1334. ALLEN, K. RADWAY. 1969. Limitations on production in salmonid populations in streams. H. R. MacMillan Lectures in Fisheries, Symp. Salmon Trout Streams 1968: 3–18.

1335. CALAPRICE, J. R. 1969. Production and genetic factors in managed salmonid populations. H. R. MacMillan Lectures in Fisheries, Symp. Salmon Trout Streams 1968: 377–388.

1336. YAMAZAKI, FUMIO, AND EDWARD M. DONALDSON. 1969. Involvement of gonadotropin and steroid hormones in the spermiation of the goldfish (*Carassius auratus*). Gen. Comp. Endocrinol. 12: 491–497.

*1337. BUTLER, D. G., W. C. CLARKE, E. M. DONALDSON, AND R. W. LANGFORD. 1969. Surgical adrenalectomy of a teleost fish (*Anguilla rostrata* LeSueur): effect on plasma cortisol and tissue electrolyte and carbohydrate concentrations. Gen. Comp. Endocrinol. 12: 503–514.

*1338. FAGERLUND, U. H. M., AND EDWARD M. DONALDSON. 1969. The effect of androgens on the distribution and secretion of cortisol in gonadectomized male sockeye salmon (*Oncorhynchus nerka*). Gen. Comp. Endocrinol. 12: 438–448.

Copies of individual reprints, if available, may be obtained from the authors or Board establishments through which the investigations were conducted.

Reprints of these articles are included in an annual volume of "Studies," composed of two parts. These are distributed to a selected mailing list. (Articles numbered 1235 to 1262 appeared in the Studies for 1968, Part 2.)

*Indicates a "Titles only" paper which will not be included in the binding.